



Ultrasound irradiation in the production of ethanol from biomass



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ABSTRACT

Ethanol produced from renewable biomass, such as lignocellulosic feedstock, is one of the alternative energy resources that can be environmentally friendly. However, physical and chemical barriers caused by the close association of the main components of lignocellulosic biomass, as well as starch, hinder the hydrolysis of cellulose and hemicellulose in lignocellulose as well as amylase and amylopectin in starch to fermentable sugars. One of the main goals of pretreatment for enzymatic hydrolysis is to increase the enzyme accessibility for improving digestibility of cellulose and starch. Ultrasound irradiation applied to cellulosic materials and starch-based feedstock was found to enhance the efficiency of hydrolysis and subsequently increase the sugar yield. Prior research conducted on applying ultrasonic technology for cellulose and starch pretreatment has considered a variety of effects on physical and chemical characteristics, hydrolysis efficiency and ethanol yield. This paper reviews the application of ultrasound irradiation to cellulose and starch prior to and during hydrolysis in terms of sugar and ethanol yields. It also addresses characteristics such as accessibility, crystallinity, degree of polymerization, morphological structure, swelling power, particle size and viscosity as influenced by ultrasonic treatment.

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Contents

1. Introduction	401
2. Ultrasound	402
2.1. Acoustic cavitation	402
2.1.1. Chemical effects	404
2.1.2. Physical effects	404
2.2. Factors of consideration in sonochemistry	405
3. Lignocellulosic biomass	406
3.1. Process of producing ethanol from lignocellulosic biomass	406
3.1.1. Structure	406
3.1.2. Conversion to ethanol	406
3.1.3. Pretreatment	406
3.2. Effect of ultrasound on physical characteristic of cellulose	407
3.2.1. Accessibility	407
3.2.2. Crystallinity	408
3.2.3. Degree of polymerization	409
3.2.4. Morphology	410
3.3. Effect of ultrasound on sugar yield	411
3.4. Effect of ultrasound on ethanol yield	412
4. Starch	413
4.1. Process of producing ethanol from starch	413
4.1.1. Structure	413

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4.1.2.	Conversion of starch to ethanol	413
4.2.	Effect of ultrasound on physical characteristics of starch	413
4.2.1.	Swelling power and disintegration	413
4.2.2.	Particle size	414
4.2.3.	Morphology	415
4.2.4.	Depolymerization	416
4.2.5.	Viscosity	416
4.3.	Effect of ultrasound on hydrolysis	418
4.4.	Effect of ultrasound on fermentation	419
4.5.	Effect of ultrasound on immobilized enzymes	419
5.	Conclusions	419
	References	420

1. Introduction

Long-term economic and environmental concerns have resulted in a great amount of research on renewable sources of liquid fuels such as ethanol and biodiesel to replace fossil fuels. Greenhouse gases from burning fossil fuels have major consequences for climate change [1]. The United States, with only 4.5% of the world's population, is responsible for about 25% of global energy consumption and 25% of global CO₂ emissions [2]. The average price of gasoline in the U.S. in 2005 was \$2.56 per gallon, while in 2011 average price had reached \$3.52 per gallon, a 37.5% increase [2]. In the last three decades, the consumption of liquid transportation fuels such as gasoline, diesel, and jet fuels in the U.S. increased by 50% [3]. The liquid transportation fuels from petroleum account for 70% of total petroleum consumption [4]. As regarding finite reserves, non-uniform distribution, volatile prices, and contribution to greenhouse gas (GHG) emissions from petroleum, development of sustainable sources to replace conventional liquid transportation fuels appears critical [5].

Biofuels, such as ethanol and biodiesel, are considered to serve not only as agents of greenhouse gas reduction but also as a means to secure an energy supply that is local, renewable and independent of a financially volatile and potentially unreliable oil market [6]. The role of ethanol as a blended gasoline product, in ratios of 5–20% by volume (v/v) ethanol has many advantages when considering the ease of adaptation to present oil infrastructure. Alcohol blended at lower fractions with gasoline can be used without major modification to modern automobile engines and burns cleaner reducing harmful emissions [7]. Higher octane ratings also improve combustion and potentially allow for higher engine efficiencies. At higher blending ratios up to 85% by volume in gasoline making so-called E85, ethanol is already adapted in the U.S. to flexible fuel vehicles. Elsewhere around the world, neat or unblended ethanol is used. According to the Energy Information Administration (EIA), the U.S. net foreign petroleum dependence peaked in 2005 at just over 60%—the same year the federal Renewable Fuels Standard (RFS) was enacted. Since then the increased use of domestic, renewable fuels has been a major force in reducing the U.S. net foreign petroleum dependence to less than 50%. With 13.9 billion gallons of domestic ethanol production, the U.S. required 485 million fewer barrels of imported oil in 2011 [8]. Other issues associated with ethanol from corn, however, such as overall sustainability due to indirect land use changes and other effects, remain controversial.

Ethanol is produced from different constituents of the raw materials [9]. Feedstock can be conveniently classified into three categories: (i) sucrose-based (e.g. sugar cane, sugar beet, sweet sorghum and fruits), (ii) starch-based (e.g. corn (maize) grain, milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (iii) lignocellulosic biomass (e.g. wood, straw and grasses) [10].

Currently, focus is on ethanol production from crops including corn, wheat, sorghum, sugar cane, as well as abundant agricultural wastes, which may in practice prove more sustainable compared to agricultural products. They may also be less expensive in comparison to conventional agricultural feedstock or purpose grown crops [9,10b].

Lignocellulosic and starch-based feedstocks require various forms of pretreatment to enhance biofuel and bioenergy production [11]. The goal of a pretreatment process is to break down and disrupt the crystalline and amorphous regions in the structure of cellulose and starch, so that the acids or enzymes can easily access and hydrolyze them [1a,10a]. Pretreatment is important to the effective conversion of feedstock, but is often the most expensive process. Substantial research and development has been directed at identifying lower cost alternatives to reduce cost [1a]. The use of ultrasound irradiation is considered one possibility and is reviewed in further detail here.

Sound waves can be categorized based on frequency into three groups: infrasound ($f < 20$ Hz), human audible sound ($20 \text{ Hz} < f < 20 \text{ kHz}$), and ultrasound ($f > 20 \text{ kHz}$) [12]. Ultrasound waves are generally associated with the frequency range of 20 kHz to 500 MHz. The interest in ultrasound irradiation and cavitation, induced by applying ultrasound to a liquid, started over 100 years ago. Thomycroft and Bamaby observed the cavitation process for the first time in 1895, when they found that the propeller of their submarine was pitted and eroded over a relatively short operating period [13]. Lord Rayleigh published the first mathematical model, analyzing a cavitation in 1917 [14]. Ultrasound irradiation to enhance reaction rates was first used by Richards and Loomis in 1927 [15]. The degradation of a biological polymer by ultrasound was published by Brohult [16] in 1937 and later, Schmid and Rommel developed the field of synthetic polymer degradation using ultrasound [17].

Because of their short wavelengths, high-frequency ($f > 1 \text{ MHz}$) ultrasound waves are useful for detection and imaging of small areas with high accuracy such as measuring velocity, in medical scanning, imaging, and treatment of dental stains, among other uses. In contrast, low-frequency waves ($20 \text{ kHz} < f < 1 \text{ MHz}$) with high intensity have the potential of producing physical and chemical changes. The utilization of low frequency, high intensity ultrasound in chemical processes is referred to as sonochemistry. A wide variety of ultrasound applications are presented in Table 1 [13,18].

Pretreatment of lignocellulosic materials and starch-based feedstock by ultrasound irradiation can enhance ethanol yield through structural deconstruction of the materials resulting from cavitation forces. The current work focuses on investigation of the effective factors of ultrasonic systems for ethanol production, the effects on physical and chemical characteristics of lignocellulosic and starch-based feedstock, and recent advances for feedstock pretreatment in biofuel applications.

2. Ultrasound

Chemical reactions require some form of energy to proceed. Chemical reactions are well known to cease as the temperature approaches absolute zero [19]. The properties of a specific energy source can determine the course of a chemical reaction [19b]. Ultrasound irradiation has its own realm of reaction conditions such as time, pressure and energy per molecule, which are determined based on inherent reaction parameters of each type of energy (Fig. 1). Ultrasonic irradiation provides rather unusual conditions in comparison with more traditional energy sources that cannot be realized by other methods [19]. Such extraordinary effects of ultrasound do not derive from a direct interaction with molecular species. Instead, sonochemistry derives from acoustic cavitation, that is the formation, growth, and implosive collapse of bubbles in a liquid [19a].

2.1. Acoustic cavitation

Acoustic cavitation is the phenomenon of the generation of micro-bubbles (cavities) in a liquid during the rarefaction, or negative pressure produced by concentrating the diffuse energy of sound [19b]. When a sufficiently large negative pressure is applied to a liquid, the increasing distance between the molecules

results in voids or cavities being created; cavitation bubbles will then be formed (Fig. 2). The required negative pressure to produce cavitation is proportional to the tensile strength of the liquid and thus depends on the type and purity of the liquid. For instance, more than 1000 atm of negative pressure is required to produce cavities in pure water whereas in tap water, a few atm of negative pressure is enough for cavitation [19b,20]. Highly soluble impurities like salt and zinc sulphate increase the surface tension because of sharing attractive forces of solute molecules. On the other hand, if the impurities added to water are much less soluble, the intermolecular force decreases with a concomitant decrease in the surface tension. An oscillating bubble can accumulate energy from the oscillations in the form of heat. With continuing energy input, the bubble grows until reaching a size (typically tens of μm) at which the void structure is no longer stable. The bubble then suddenly collapses resulting in the rapid release of the stored energy with a heating rate of $> 10^{10} \text{ K/s}$. This transient

Table 1
Applications of ultrasound [13,18].

Ultrasound waves	Low frequency ultrasound (20 kHz–1 MHz)	High frequency ultrasound (1–10 MHz)
Low intensity	Sonophoresis	Medical diagnosis Chemical analysis Food quality assessment Nondestructive tests Massage therapy Drug delivery
High intensity	Welding Cleaning Cell disruption Lithotripsy Engineering applications Sonochemistry	

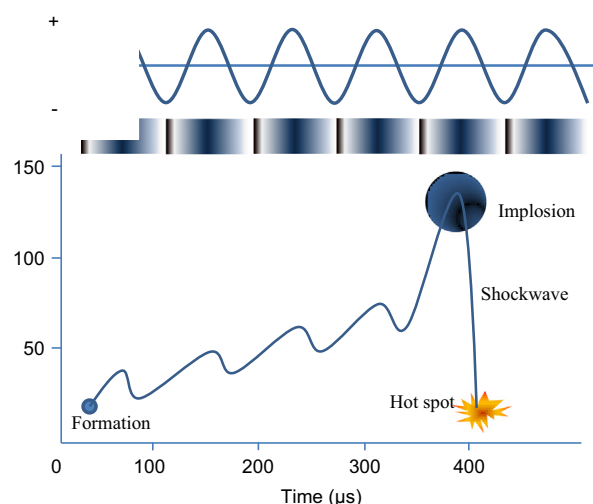


Fig. 2. Schematic representation of a sound wave, transient acoustic cavitation and the corresponding pressure fluctuations: Bubble radius (μm) versus time (μs).

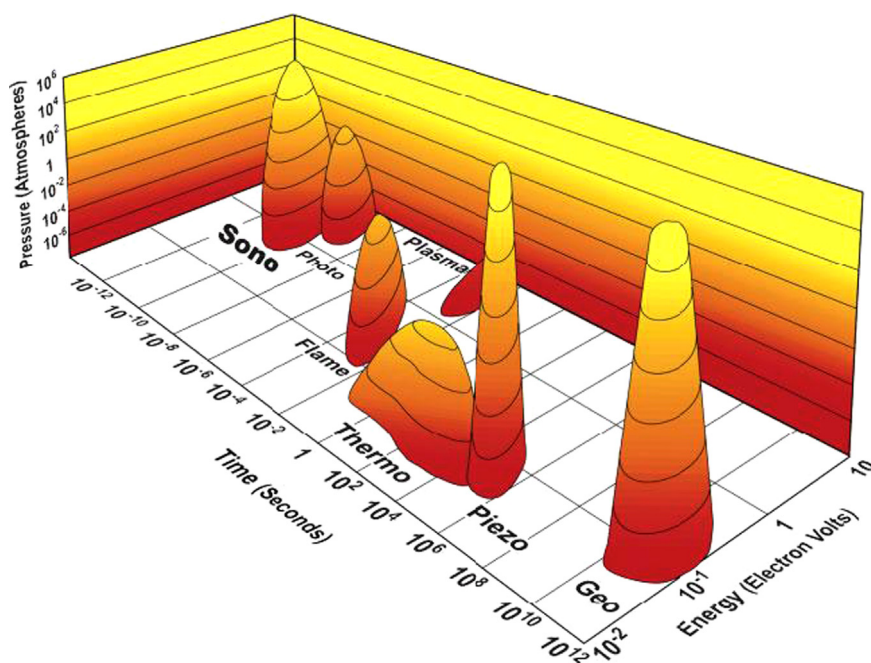


Fig. 1. Islands of chemistry as a function of time, pressure, and energy (reproduced from Ref. [19b]).

cavitation implosion is highly localized with associated temperature of roughly 5000 K and pressure of about 1000 bar [19b]. Dissolved gases in the liquid result in so-called weak spots and the number of bubbles produced during the rarefaction cycle is proportional to the density of such weak spots [21].

In the literature, it is claimed that ultrasound cannot affect chemical reactions when there are no dissolved gases in the fluid, when the sound intensity is not big enough to overcome the cavitation threshold (a minimum acoustic pressure necessary to initiate bubble growth) of the fluid [22], or when the reactants are not volatile enough to enter the cavitation bubble as vapor during its formation [13]. Indeed there are two agents for bubble formation involving pre-existing bubbles in the liquid and gas trapped in solid particles present in the liquid [21a]. A larger bubble can be created as trapped gas exiting the crevices of a particle under the influence of a motivating force (such as that developed during exposure to ultrasound) coalesces with pre-existing bubbles. The bubble formed may continue to grow until it reaches a critical size, known as its resonance size, which depends on the applied frequency of the sound field [19–21].

The cavity expansion produced by high-intensity ultrasound sometimes occurs so quickly during the negative pressure cycle that the positive pressure in the next cycle is not able to reduce the size of the cavity. However, in the case of low-intensity ultrasound, the size of the cavity oscillates in phase with the expansion and compression cycles. The surface area of this bubble will be comparatively larger during expansion cycles in comparison with compression cycles. The bubble at its critical size absorbs energy more efficiently from ultrasound and grows more rapidly. After a very rapid growth, the efficiency of energy absorption declines, the bubble pressure can no longer resist the hydrostatic pressure of the liquid, and the cavity collapses with an implosion of the bubble [19b,20]. In summary, the phenomenon of cavitation consists of three distinct steps: (1) bubble nucleation (formation), (2) rapid growth and expansion to a critical size during alternating cycles of compression-rarefaction, and (3) implosion and violent collapse of the bubble in the liquid [23].

The critical size of cavitation bubbles is inversely proportional to the frequency of sound waves. For example, the radii of bubbles produced by ultrasound at a frequency of 20 kHz were within 100–170 μm , while at 1 MHz the radius was about 3.3 μm [24]. A simple and approximate relationship between the resonance radius of the bubble and the frequency, which is given by [21a,25].

$$f \times R \approx 3 \quad (1)$$

where f is the frequency (Hz) and R is the bubble radius (m).

The bubbles generated in the fluid subjected to 20 kHz ultrasound frequency are relatively large and their collapse produces strong shockwaves, which are useful for mechanical shearing applications such as emulsification and pretreatment of starch and lignocellulosic materials [21a,26]. Sound waves of 100 to 1000 kHz generate much smaller bubbles, which induce a higher increase in temperature upon collapse useful for sonochemical purposes [26b]. Above 1 MHz frequency, cavitation effects are much weaker and do not substantially enhance chemical reactions. However, ultrasound waves have some applications in this frequency range such as the gentle cleaning of electronic parts, the nebulization of liquids to produce fine sprays and imaging for medical and industrial purposes [26a].

Gas bubbles in liquids subjected to the sound field show several behaviors, which are described in detail by Leong et al. [21a]. Apfel has produced a series of charts based on theoretical equations to predict the bubble growth thresholds [27]. An example of these charts is shown in Fig. 3. The figure illustrates the separated regions of different types of cavitation activity. The bubbles in the region A are under inertial control so that the bubble growth only

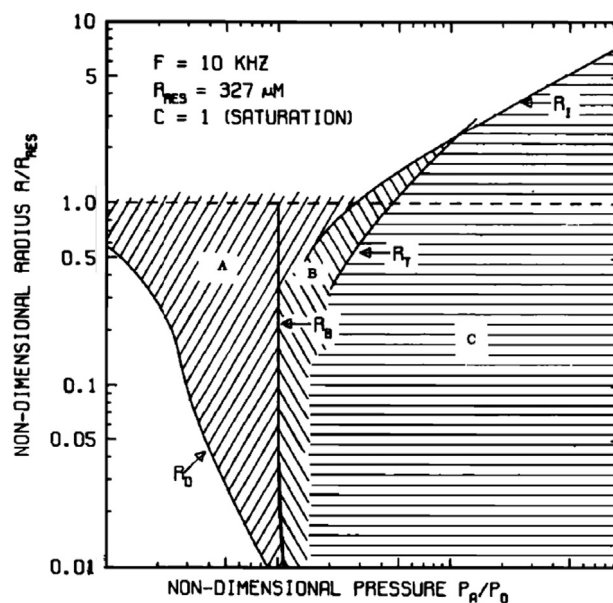


Fig. 3. Cavitation prediction chart for a 10 kHz system in a 100% gas saturated system (reproduced from Ref. [27]). Region A is related to a bubble under inertial control, B the region for growth by rectified diffusion and C the region for transient cavitation.

occurs via rectified diffusion (growing the bubbles with time over several acoustic cycles). The bubbles in this region undergo more violent behavior and collapse once their sizes reach resonance ($R/R_{\text{res}}=1$). In region B, the bubbles grow by rectified diffusion although they are not initially transient. After fragmentation, microbubbles generated may be in region C, and, then may go transient. Region C is the transient region for cavitation and the border with region B indicates the transient threshold [26a].

So far, there are four theories proposed to explain acoustic cavitation events: hot-spot, electrical, plasma discharge and supercritical theories. The “hot-spot” theory is based on the claim that a pressure of thousands of atmospheres is generated with a temperature of up to 5000 K during the violent collapse of a bubble [28]. For perspective, the cavitation produced by using ultrasound irradiation can produce the temperature of the sun’s surface, the pressure of deep oceanic trenches, and the cooling rate of molten metal splatted onto a liquid-helium-cooled surface [19b]. It is interesting to note that a light emission of more than 100 photons per flash can be achieved because of such a high temperature [19b,20]. This theory suggests that sonoluminescence basically results from blackbody radiation from the gas within the bubble during adiabatic collapse [21]. It appears that most studies in sonochemistry have adopted the hot-spot concept to explain experimental results.

Some scientists believe that the extreme conditions occurring with fragmentation of bubbles are due to the intense electrical fields [28,29]. The electrical theory suggests that during bubble formation and collapse, enormous electrical field gradients are generated which are sufficiently high to cause bond breakage and chemical activity [28]. Although the plasma theory also suggests that the collapse of bubbles is due to intense electrical fields, the process seems not to involve a true implosion. The theory attributes the origin of cavitation to corona-like discharges caused by a fragmentation process. This view is supported by drawing numerous analogies between sonochemistry and corona chemistry and indicating the formation of micro-plasmas inside the bubbles [28,29]. Finally, the supercritical theory claims that there is a layer in the bubble-solution interface, where temperature and pressure may be beyond the critical conditions of water (647 K,

22.1 MPa). The conditions may have physical properties intermediate between those of a gas and a liquid. This theory suggests that supercritical water is obtained during the collapse of cavitation bubbles generated within the water subjected to ultrasound irradiation.

2.1.1. Chemical effects

Acoustic cavitation in fluids can induce chemical and physical effects. The chemical effects of ultrasound irradiation have been explored for many years, nearly all in water [13,18,19,30]. In environmental engineering, chemical effects of ultrasound irradiation are related to degradation of organic pollutants using pyrolysis and radical reactions [18,20,23,30]. Irradiating ultrasound waves within aqueous liquids generates free radicals.

In the structural model of the hot-spot, three regions for the occurrence of chemical reactions are postulated: (i) a hot gaseous nucleus; (ii) an interfacial region with radial gradient in temperature and local radical density; and (iii) the bulk solution at ambient temperature [28]. Free radicals are produced within the collapsing bubble, at the interface of the bubble, and in the surrounding liquid. Because of the harsh conditions generated on bubble collapse, bond breakage and/or the dissociation of the water and other vapors and gases, the formation of free radicals will happen within the center of the bubble. The high temperature and pressure produced during bubble cavitation provide the activation energy required for the bond breakage [28]. The formation of free radicals in water subjected to ultrasound has been particularly well studied. Primary products in sonicated water are H^\bullet and OH^\bullet radicals [13,19a]. The radicals produced can be recombined to return to their original water form or combined to generate H_2 and H_2O_2 . Meanwhile, it is also possible to produce HO_2^\bullet radicals by combination with O_2 . These strong oxidants and reducers can be utilized for various sonochemical reactions in aqueous solutions [19a]. However, the radicals created either react with each other to form new molecules and radicals or diffuse into the bulk liquid to serve as oxidants [13,28]. The second reaction site is the liquid shell immediately surrounding the imploding cavity, which has been estimated to heat up to approximately 2000 K during cavitation. It seems that both combustion and free radical reactions occur within the layer surrounding the hot bubble [20,28]. Although some subsequent reactions generating intermediates may occur in the bulk liquid, no primary sonochemical activity takes place there. A small number of free radicals produced within bubbles or at the interface may move into the bulk liquid phase and react with the substrate through secondary reactions to form new products [28].

2.1.2. Physical effects

Physical effects are involved in pretreatment of starch and lignocellulosic materials using micro-jet and shock waves. Generally, chemical and physical effects occur simultaneously. However, physical effects can be much more violent in heterogeneous systems involving liquid-solid systems, like enzymatic hydrolysis of lignocellulosic materials [18,26,30].

A deformation of the bubble during its collapse occurs because of the asymmetry of the environment near the interface. This deformation, which is self-reinforcing, sends a fast moving stream of liquid through the bubble at the surface with velocities at least 100 m/s. This micro-jet impact leaves behind characteristic microscopic pitting in the surface of solids [19b]. In addition, shock waves are also created which are able to produce microscopic turbulence within interfacial films surrounding nearby solid particles, also termed micro-streaming [13,26a]. This phenomenon increases the transfer of mass across the solid particles, leading to an increase to the intrinsic mass transfer coefficient [13,26b].

Although the existence of both mechanisms, micro-jet and shock waves, has been established, their relative importance is a matter of debate and probably depends on the method by which cavitation is produced [19b]. For instance, effects of micro-jets produced by the bubble collapse depend on a surface several times larger than resonant bubble size. So, it is expected that for solid particles smaller than 200 μm , micro-jets can be created with ultrasonic frequencies of 20 kHz. In these conditions, the shock waves produced by homogeneous cavitation can be caused by creation of high velocity inter-particle collisions [19a].

The diffusion transport of enzyme macromolecules toward the surface of a solid substrate like lignocellulose and starch could be certainly enhanced by agitation of the processing solution [26a]. However, it is also found that mechanical agitation is not a highly effective mixing method for the immediate border layer of liquid at a solid-liquid interface where the enzyme reactions actually takes place [18]. A schematic of varied velocities in accordance to the layers of liquid concentrically surrounding the solid particle is shown in Fig. 4 [26a]. The first layer next to the particle is immobile and the velocity quickly increases in the next layers to the maximum constant velocity as achieved by the power of agitation of the bulk solution. Because the first layer (immediate adjacent layer) is basically motionless, it seems that the only available mass transport mechanism to reach the substrate surface is diffusion. It is notable that the diffusion in the case of large macromolecules like lignocellulose and starch by using the simple mechanical agitation is quite inefficient [26]. The shock-waves produced by collapse of bubbles on and near the surface of this substrate (e.g. corn stover particles) are more effective within this first layer of liquid where the enzymatic reaction occurs. This forceful stirring/mixing leads to a high improvement in the supply of enzyme macromolecules to the surface of a substrate.

Shear forces of shock-waves are approximately calculable. As mentioned above, the velocity of the fluid (with viscosity η) at the surface of solid particle is negligible and the velocity V of the fluid at the location (x_0) produces the stress σ . Assuming that the collapse of the bubble is very near to the particle surface, this stress created by shear forces of shock-waves is estimated by the following equation [26b]:

$$\sigma \approx \frac{\eta V}{x_0} \quad (2)$$

According to Eq. (2), a bubble collapse with velocity of 200 m/s at $x_0 = 1 \mu\text{m}$ from the particle surface generates a shear stress equal to $2 \times 10^5 \text{ Pa}$ in water [26b].

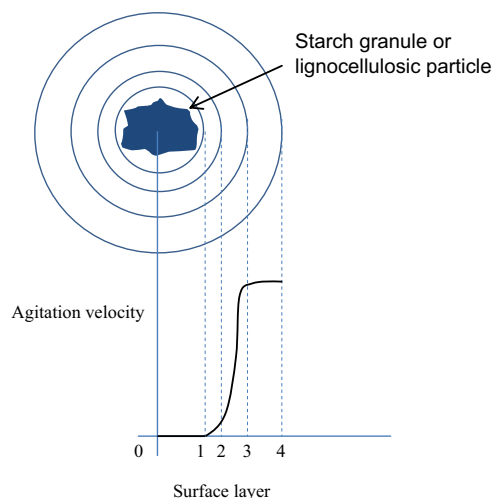


Fig. 4. Schematic distribution of the velocities of the layers of liquid concentrically surrounding the solid particle (adapted from Ref. [26a]).

Most cavitation bubbles generated close to the particle surface provide an important additional benefit termed “opening up” or pore creation resulting from mechanical impacts generated by micro-jets [13,26a,30].

The effective range of micro-jets in “opening up” the surface is proportional to the bubble diameter. The stress generated by a micro-jet can be calculated by the following equation [26a]:

$$\sigma \sim \frac{2\gamma}{R} 0.157 \left(\frac{R}{x_0} \right)^3 \quad (3)$$

where γ is the surface tension of the cavitation bubble and R is the bubble radius. According to Eqs. (2) and (3), at low x_0 the stress

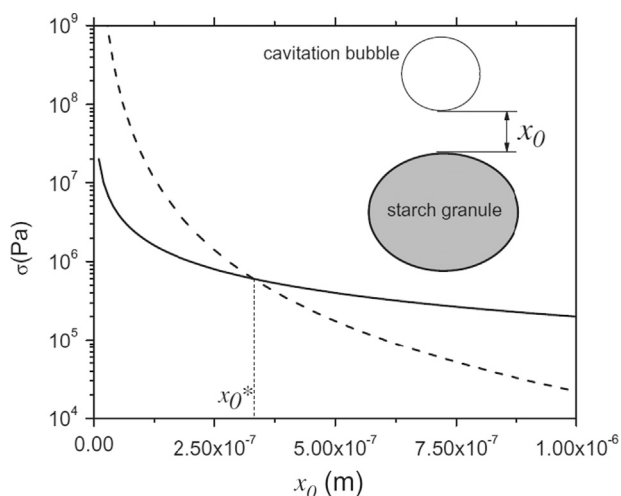


Fig. 5. Shear stress (solid line) and micro-jet stress (dashed line) as a function of the cavitation bubble to starch granule or lignocellulosic particle distance x_0 (reproduced from Ref. [26b]). Shear stresses are calculated based on $\mu = 1 \times 10^{-3}$ Pa s and $v = 200$ m/s; and micro-jet stresses are calculated using $R = 1 \times 10^{-6}$ m, $\gamma = 70 \times 10^{-3}$ N/m. x_0^* is the critical cavitation bubble to starch granule or lignocellulosic particle distance at which the shear and micro-jet stresses are equal. Schematic represents stress as a function of the cavitation bubble to solid particle distance x_0 .

caused by a micro-jet is higher than that generated by the shear forces, which is shown in Fig. 5. However, at distances more than x_0^* , as the transition point, the shear stress plays a dominant role in physical effects of cavitation [26]. These stresses induced by micro-jets and shock-waves in heterogeneous systems make the effects of cavitation several hundred times greater than that in homogeneous systems [26b].

2.2. Factors of consideration in sonochemistry

Many studies have been carried out to find and investigate the effective factors on the outcome of a sonochemical reaction, which can be categorized as physical, chemical and macroscopic effects [12,18,28]. A summarized collection of the effects is presented in Table 2. The cavitation dynamics are strongly affected by the physical properties of the liquid and gases dissolved including bulk temperature, viscosity and surface tension [18]. In addition, the characteristics of the ultrasound waves applied, like intensity and frequency, are also effective. Many of these physical effects can be predicted using a single cavity dynamics model [12,13].

As mentioned above, upon bubble collapse, the molecules present within or around the bubble can decompose and form radicals. Since, increasing temperature varies in accordance to the different reaction zones, the position of a reactant plays an important role in cavitation [18,20]. Thus, a volatile reactant is susceptible to thermal decomposition inside the bubble, whereas a non-volatile reactant can decompose just in the relatively cold liquid shell surrounding the bubble or by attack from primary radicals. Thus, the number and type of radicals generated are strongly dependent on the chemical characteristics of the reactants [18,28].

The previously discussed physical and chemical effects are associated with processes occurring on just a single bubble, whereas ultrasound equipment generates a large cloud of bubbles simultaneously [18]. The interaction among the neighboring bubbles induces shape distortions, reducing the efficiency of the cavitation collapse [31]. This negative effect is reinforced by the

Table 2
Factors influencing ultrasonic cavitation.

Factors	Influence on cavitation phenomena
Presence and nature of dissolved gases	Presence of gas in the fluid can lower the cavitation threshold and subsequently reduces the intensity of the shock wave generated [12]. Particle matter such as cellulose and starch can bring some trapped vapor nuclei in their crevices and recesses, which causes to increase the number of cavitation but reduce the cavitation effects [12,28,63]. Cavitation is also influenced by several properties gas including heat capacity ratio, thermal conductivity and solubility. In general it is known that the bubble collapse is an adiabatic process [13,20]. Thus a higher temperature and pressure are expected with gases of high heat capacity ratio. Lower thermal conductivity and higher solubility also should provide better cavitation [20].
Ambient temperature	The cavitation threshold should increase as temperature of bulk solution decrease, because it is directly in proportional to solvent vapor pressure [12,20]. In boiling point of solvent also a large number of cavitation bubbles is concurrently produced which act as a barrier to sound transmission and nullify the effect of ultrasound energy [12,20].
Ambient pressure	With increase in the ambient reaction pressure, the sonochemical effects will increase because of the decrease in the vapor pressure of the mixture. Decreasing the vapor pressure results in enhancing the intensity of the implosion with a subsequent increase in the ultrasound energy generated upon cavitation [12,20].
Choice of solvent	The sonochemical process like ultrasonic treatment is also influenced by solvent properties such as vapor pressure, viscosity, surface tension and density. Liquids with high vapor pressure and low density can produce favorable conditions for cavitation. In contrast, solvents with higher viscosity, surface tension and density showed poor cavitation efficiency [13,20].
Ultrasonic frequency	The ultrasound frequency has a significant effect on the cavitation process because of its influence on the critical size of the cavitation bubble. Lower frequency ultrasound can produce more violent cavitation, resulting in higher localized temperatures and pressures at the cavitation site as well as more effective shock waves. Higher frequencies may generally increase the number of free radicals in the fluid because, although cavitation is less violent, there are more cavitation events and thus more opportunities for free radicals to be produced [13]. Aside from that the shortened bubble lifetime can increase the amount of free radicals which are able to escape from inside the cavitation region to the bulk mixture, where they facilitate the bulk reaction. It is contended that the optimum frequency is dependent on whether intense temperatures and pressures as well as violent shock waves are required (thus enhanced by lower frequencies) or if the rate of single electron transfer is more important (enhanced by higher frequencies) [12,13].
Acoustic power	According to many authors, as the power applied to the reaction mixture increases, the rate of the reaction increases to a maximum and then decreases with a continued increase in power [13]. A possible reason for the observed decrease at ultrasound powers more than the optimum value is the formation of a dense cloud of cavitation bubbles near the probe tip which acts to block the energy transmitted from the probe to the fluid [12,13]. The optimum power level should also depend on the operating frequency [13].

self-organization and clustering of bubbles under the influence of acoustic forcing on and between the bubbles [18,31].

3. Lignocellulosic biomass

3.1. Process of producing ethanol from lignocellulosic biomass

3.1.1. Structure

Lignocellulose is the primary building block of plant cell walls, which is basically composed of cellulose, hemicellulose, and lignin along with lower amounts of pectin, protein, ash, and extractives as soluble non-structural materials such as nitrogen materials, non-structural sugars, chlorophyll, and waxes [1a,10b]. The amount of each one of these constituents varies in various plant species. For instance, hardwood and softwood have greater amounts of cellulose, whereas in leaves there is more than 80% hemicellulose (Table 3).

Cellulose, as the principal carbohydrate component in plant cell walls, is found in an organized fibrous structure [1a,5]. This macromolecule consists of D-glucose subunits linked to each other by β -(1,4)-glycosidic bonds [5,10b]. The branches of fermentable D-glucose can be separated from cellulose through the action of either acid or enzymes breaking the β -(1,4)-glycosidic linkages. The cellulose polymers are basically linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into micro-fibrils [1a]. These micro-fibrils can be further aggregated in forming fibrils and then fibers [5]. Cellulose fibers are imbedded in a matrix of hemicellulose and lignin polysaccharides [5]. Although, cellulose is found to have a highly crystallized structure as the major proportion of cellulose, a smaller percentage of unorganized cellulose chains also form amorphous regions [1a,10b]. Crystalline regions are much more difficult to hydrolyze to glucose in comparison to that of the amorphous regions [5,11].

The main difference between hemicellulose and cellulose is the fact that hemicellulose has branches with short lateral chains consisting of different sugars. These mono-saccharides connected to hemicellulose consist of both six-carbon and five-carbon sugars including arabinose, galactose, glucose, mannose, xylose, and other species [5]. It is believed that xylose has the largest proportion in hemicellulose [5,11]. Either a homo-polymer or a hetero-polymer with short branches connected by β -(1,4)-glycosidic and occasionally β -(1,3)-glycosidic bonds basically form the backbone of hemicellulose [1a]. In addition, there is some degree

of acetylation in the structure of hemicellulose, for instance, in heteroxylan. The polymers existing in hemicellulose can be more easily hydrolyzed compared to cellulose because polymers of hemicellulose cannot be compacted even when they cooperate with cellulose chains to form crystals.

Lignin is also another macromolecule present in the primary cell wall and contains cross-linked polymers of phenolic monomers. Lignin imparts structural support, impermeability, and resistance against microbial attack [1a,10b]. There are three phenyl propionic alcohols as monomers of lignin including coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol) [1a,5]. However, lignin contains no sugars, and is not convertible to ethanol using the current yeast fermentation technologies [5]. Herbaceous plants such as grasses generally have the lowest contents of lignin, whereas the highest lignin contents belong to softwoods among lignocellulosic materials (Table 3).

3.1.2. Conversion to ethanol

Hydrolysis is used to break down cellulose into its components, which are fermentable sugars [32]. Two major types of hydrolysis processes are widely introduced: acid hydrolysis and enzymatic hydrolysis [5]. Concentrated or diluted acids are generally used in acid hydrolysis of cellulosic biomass resulting in problems with acid recovery, equipment corrosion, and decomposition of product sugars. Enzymatic hydrolysis does not have these problems, but the efficiency of this method is very low compared to that of acid hydrolysis, leading to increasing the production cost [11]. It is concluded that cellulosic biomass needs extensive pretreatment to increase the efficiency of enzymatic hydrolysis [5,32]. The produced sugars in the hydrolysis step can be fermented by bacteria, yeast or filamentous fungi to produce ethanol. The enzymatic hydrolysis and fermentation steps can also be combined in a step called simultaneous saccharification and fermentation (SSF) [33]. The ethanol purified by distillation and molecular sieves or other separation techniques as the last step will be ready to be used as a fuel, either neat or blended with petrol [33].

3.1.3. Pretreatment

As mentioned above, the pretreatment of lignocellulosic materials is required to improve the efficiency of cellulose hydrolysis to its constituent sugars. Some of important factors relative to hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, crystallinity of cellulose fibers, and content of both lignin and hemicellulose [1a,10b]. The accessibility of cellulase enzymes and acids to cellulose is inversely proportioned to amount of lignin and hemicellulose in these materials. Thus, lignocellulose with high lignin and hemicellulose has low efficiency of hydrolysis [11]. The lignin and hemicellulose must be disrupted as must the crystalline structure of cellulose, which are the primary purposes of pretreatment. An ideal pretreatment can also increase the surface area of cellulose and make it more accessible to enzymatic hydrolysis, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly and with greater yields [5]. In summary, pretreatment can significantly improve the hydrolysis process by disruption of lignin and hemicellulose, reduction of cellulose crystallinity, and enhancement of porosity (Fig. 6) [1a,5].

Pretreatment methods of lignocellulosic materials can be roughly classified as physical, physicochemical, chemical, biological, electrical, or a combination of these [1a,5,10b]. Several key properties to take into consideration for low-cost and advanced pretreatment processes are summarized in Table 4 [10b].

Table 3
Cellulose, hemicellulose, and lignin contents in common agricultural residues and wastes [1a].

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15		
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12
Swine waste	6	28	na

3.2. Effect of ultrasound on physical characteristic of cellulose

3.2.1. Accessibility

The schematic substrate shown in Fig. 7(a) assists in better understanding the accessibility concepts and their roles in hydrolysis [34]. It should be noted that the schematic has not been drawn to scale and in reality, the accessible and non-accessible portions can be mixed with each other.

During hydrolysis, the enzymes are adsorbed onto either the hydrolysable or non-hydrolysable (but accessible) parts of the substrate. They can transfer between the two parts via diffusion on the substrate (with no hydrolysis) [10b]. The ratios of accessible to total and hydrolysable to accessible will decrease as cellulose is hydrolyzed. The accessible fraction of cellulose can be experimentally specified by measuring its maximum

adsorption capacity [34]. Kinetic studies have been conducted following the design shown in Fig. 7(b), where enzymes are washed off at a particular conversion level. Based on these kinetics, the changes in accessibility ($[E]_{ads,max}$), reactivity (k), and hydrolysability (a) can be determined (Fig. 7(b)) [34]. The linear trend in the plot of $V_0 - [E]_{ads}$ is also in accordance with Eq. (4).

$$\text{Rate} = k \times S \times [E]_{ads} \times f \quad (4)$$

At low enzyme concentrations and short reaction times, the hydrolysis rate is proportional to the adsorbed enzyme fraction [34]. As a result, increasing accessible fraction of cellulose will enhance the hydrolysis rate for a constant enzyme concentration [5].

Ultrasound irradiation is able to crack the cell wall and dislocate the secondary wall of the middle layer, leading to an increase in accessibility of cellulase to cellulose fibers [35]. The ultrasound waves can cause the surface erosion of cellulosic materials, which is due to cavitation collapse in the surrounding liquid. Low frequency of ultrasound around 20 kHz can affect even more surface disruption because low frequency ultrasound waves can produce more violent cavitation [35,36].

Aimin et al. [37] found no significant change in crystallinity of cellulose in their study and concluded that the significant increase in accessibility could mainly be due to the morphological structure changes brought on ultrasound irradiation. Thus, it is accepted that the higher efficiency of the ultrasound-assisted extraction can be explained by mechanical action of the ultrasound waves on the cell walls leading to an increased accessibility [38]. It was also concluded that ultrasound can enhance not only the accessibility of the substrate for enzyme adsorption but also desorption of the inactively adsorbed enzyme which will cause reactivation of enzyme leading in enhanced hydrolysis efficiency [39].

Although most studies have concluded that the ultrasound irradiation could enhance accessibility of cellulose, in experiments of Yu et al. there was no obvious size reduction in rice hull subjected to ultrasonic irradiation (250 W, 40 kHz) even after 60 min [35]. They also reported that there were no significant changes in the main compositions (cellulose, hemicellulose, and lignin) under ultrasound irradiation. However, it is found that ultrasound waves can strongly affect degradation and removal of hemicellulose and lignin if used along with the alkali pretreatment

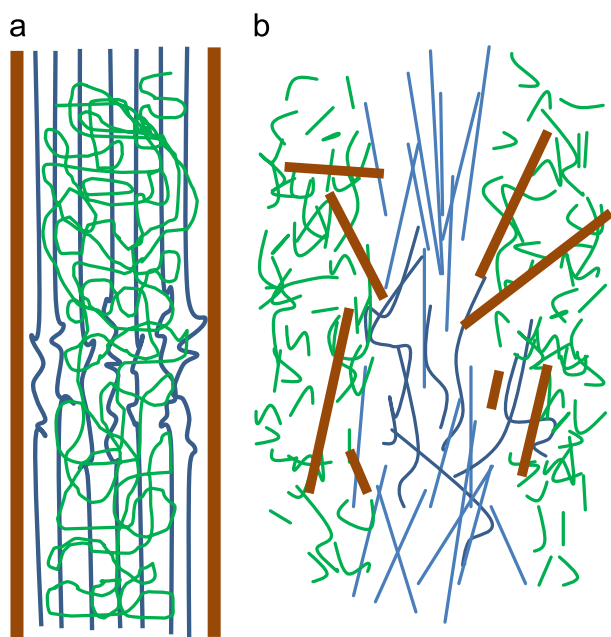


Fig. 6. Schematic of the role of pretreatment in the conversion of biomass to fuel (adapted from Ref. [1a]). (a) Before pretreatment and (b) after pretreatment.

Table 4

Key factors for effective pretreatment of lignocellulosic biomass [10b,47,117b,c].

Factors	Effects of the factors on lignocellulosic pretreatment
High efficiency for multiple crops, sites ages, harvesting times	Some pretreatments have been considered to be better suited for specific feedstock. For instance, ammonia recycling percolation (ARP) can significantly reduce the lignin content of agricultural residues but cannot be a suitable alternative for processing recalcitrant substrate such as softwoods
Highly digestible pretreated solid	A suitable pretreatment should be able to provide highly digestible cellulose; for example with yields higher than 90% in less than five days and enzyme loading lower than 10 FPU/g cellulose
No significant sugar degradation	Pretreatment should not significantly result in sugar degradation, so that a yield close to 100% of fermentable cellulosic and hemicellulosic sugars should be achieved through the pretreatment step
Minimum amount of toxic compounds	A very harsh pretreatment can generate toxic components resulting from partial hemicellulose degradation, which could affect the proceeding hydrolysis and fermentation steps. The toxic components are dependent on raw material and severity of pretreatment
Biomass size reduction not required	Size reduction by milling or grinding the raw material before pretreatment requires much energy, resulting in increase of production cost
Operation in reasonable size and moderate cost reactors	Pretreatment reactors should be low in cost and high in efficiency
Non-production of solid-waste residues	The chemicals generated during cellulose hydrolysis in preparation for subsequent steps should not present processing or disposal challenges
Effectiveness at low moisture content	Materials at high dry matter content require lower energy during pretreatment
Obtaining high sugar concentration	An adequate ethanol concentration can be achieved, provided that the concentration of sugars from the coupled operation of pretreatment and enzymatic hydrolysis is above 10%
Fermentation compatibility	The pretreatment should be compatible with the type of organism able to ferment
Lignin recovery	Lignin and other constituents should be recovered to be converted into valuable coproducts
Minimum heat and power requirements	Requirement of heat and power for pretreatment should be low to reduce the production cost

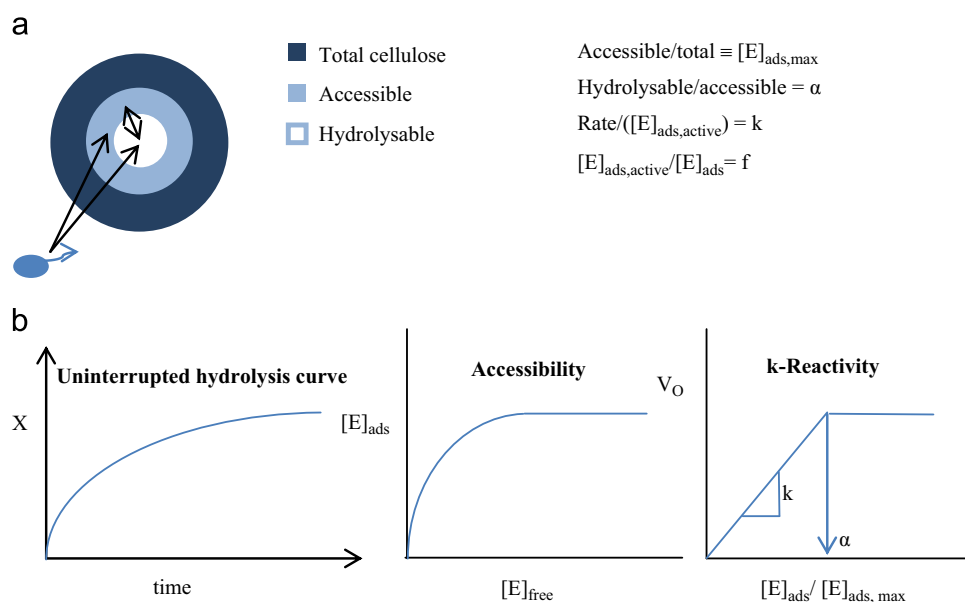


Fig. 7. (a) Schematic representation of cellulose substrate with total, accessible, and hydrolysable cellulose. Arrows indicate parts of the substrate onto which celluloses can adsorb, and between which they can change states. (b) Experimental design (V_O is the initial rate measured in terms of glucose produced in 10 min) (adapted from Ref. [34]).

[38,40]. It is reported that the removal rate of the lignin can be enhanced by 23% and the degradation rate of the hemicellulose can be increased by 12% in samples of alkali pretreated feedstock subjected to ultrasound waves with a power of 80 W and frequency of 4 kHz for 1 h. It is an interesting advantage that the slight shock and the cavitation collapse in alkaline solutions not only enhance the lignin removal rate, but also greatly increase the hemicellulose degradation rate. The dissolution of hemicellulose improves the accessibility of cellulosic materials in pretreatment, resulting in enhancement of saccharification rate of enzymatic hydrolysis [40].

Accessibility of cellulose can be quantitatively measured by determination of the size of pores distributed on the cellulose surface via the water retention value (WRV) [5]. Wojciak and Pekarovicova [41] also reported an increase in specific surface area of cellulosic fibers after ultrasonic pretreatment. Similar results were found by Wang et al. [42]. They reported that after treatment with the ultrasound waves, specific surface area and WRV increase which implied the increase of accessibility. Wang et al. [43] also found that the specific surface areas of microcrystalline cellulose (MCC) increased dramatically after 5–20 min ultrasonic treatment with a power of 500 W and a frequency of 20 kHz.

Increasing the ultrasound intensity can cause a larger accessible surface area. It seems that the positive effect of ultrasound intensity on accessibility is due to the increase of smaller particles and deeper damage of surface structure [43]. It was reported that the accessibility of cellulose increases from 73% to 119% of WRV, with increasing ultrasound (400 W, 23–25 kHz) treatment time in levels of 0 s, 90 s, 180 s, 360 s and 720 s [37]. Zeng et al. [44] found that increasing accessibility of cellulose is directly proportional to the time of ultrasound pretreatment between 0 s and 150 s. The absorbency of cellulose fiber of ultrasound pretreated samples (150 s with power of 500 W) increased from 1.4 g/g to 2.1 g/g, which shows a significant increase in accessibility. As the time of ultrasound treatment progresses beyond some optimum, the trend in accessibility will begin to decline. According to a study conducted on microcrystalline cellulose (MCC), at a constant ultrasonic power, the specific surface area reached its peak value when the treating time was 15 min. MCC pretreated by ultrasound irradiation of 500 W and 20 kHz for 15 min achieved a maximum increase in specific surficial area from 0.3 m²/g to 1.3 m²/g (Fig. 8)

[42]. Wang et al. [43] believe that when the treating time continually increases, the “hot-spot” effects generated from the collapse of ultrasonic cavitation bubbles can cause the collision and aggregation between particles, resulting in a steady decline of specific surface area.

3.2.2. Crystallinity

Cellulose in biomass is found in both crystalline and amorphous forms. The major proportion of cellulose is related to crystalline form, whereas unorganized cellulose chains, which form amorphous cellulose, comprise just a small percentage of cellulose [1a]. The crystalline fraction of cellulose has been considered as an important factor in the hydrolysis rates of relatively refined cellulose substrates [10b,11]. Crystallinity is measureable based on the percentage of crystalline region within the total structure, referred to as the crystallinity index (CrI) [5].

High-power ultrasound has the potential to increase the pore volume and to reduce the crystallinity of cellulosic biomass [11]. In a study conducted by Wang et al. [43], the degree of crystallinity of MCC after ultrasonic treatment negligibly decreased from 62% to 61%, when the ultrasonic power increased from 0 W to 200 W. However, with increasing ultrasonic power from 200 W to 700 W, a significant decrease in the degree of crystallinity of MCC was observed. It should be noted that although the degree of crystallinity decreased to 54.6% at 700 W for 15 min, the crystal size showed little change under this condition [43]. In another study it was found that both the degree and size of crystalline cellulose did not change so much after ultrasound treatment under 400 W for 12 min [37]. Other researchers also have verified that basically, the crystallinity degree and crystalline size of the treated celluloses will not change under ultrasound power below 200 W [38,45].

It seems that the treatment time of cellulosic materials using low-power ultrasound irradiation does not significantly affect the crystallinity of cellulose [43]. Generally, there is no linear relation between crystallinity and treatment time in the different powers of ultrasound waves [37,43,44]. Although the ultrasound power is a more effective factor than time in the ultrasonic treatment of cellulose, crystallinity of cellulose does not linearly decrease in accordance with increasing power of ultrasonic. This claim is supported by the relevant theory of sonochemistry that cavitation

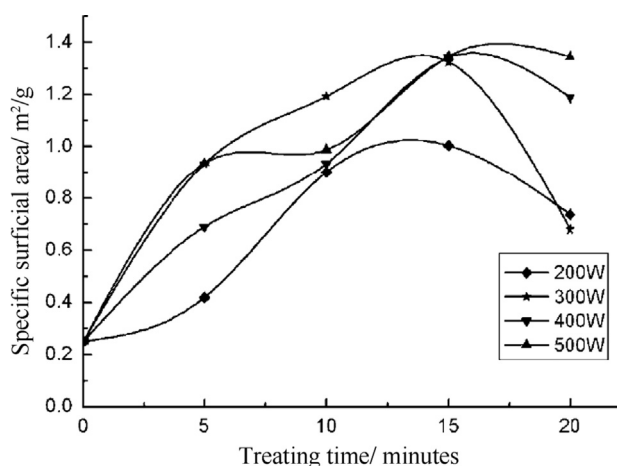


Fig. 8. The influence of ultrasound irradiation of 20 kHz for 15 min on specific surface area of MCC (reproduced from Ref. [42]).

intensity has a maximum value at the proper power of ultrasound waves [44].

Although reducing the crystallinity of cellulose only occurs with high-power ultrasound, it is found that ultrasonic-assisted alkali pretreatment effectively destroys the intermolecular hydrogen bonding of lignocellulose and as a result, decreases crystallinity of cellulose [40,46]. Reducing crystallinity under this condition can occur even with a power of 80 W. The results of the study conducted by Sun et al. [38], also revealed that the total crystallinity index of celluloses of sweet sorghum bagasse, which was defined based on the absorbance ratio, were 0.83 and 0.70 for untreated and ultrasonic-assisted-NaOH treated, respectively. The lower crystallinity and higher amorphous fraction of cellulose are due to alkali with ultrasound pretreatment [46]. Ultrasound irradiation can more effectively decompose lignocellulose with the weaker intra- and intermolecular hydrogen bonding caused by alkali pretreatment.

Using ultrasound irradiation in ionic liquids (IL) pretreatment can also significantly reduce crystallinity, which in turn contributes to an increased rate of enzymatic hydrolysis of cellulose [32,47]. IL pretreatment combined with ultrasound irradiation at a power of 35 W reduced cellulose crystallinity of kenaf more effectively than IL pretreatment with heating. The CrI values were 49.4%, 38.8% and 31.5% for the untreated sample, sample pretreated in EmimOAc for 15 min with heating and kenaf powder pretreated in EmimOAc for 15 min with ultrasound irradiation at a frequency of 24 kHz and emission power of 35 W, respectively [32]. Zeng et al. [44] also assayed the novel ultrasonic-assisted with polyethylene glycol (PEG) pretreatment method to decrease the crystallinity of cellulose fiber. PEG was used as a swelling reagent in their experiments, which could act as the pore-forming agent. Thus, a very small quantity of PEG can be useful in improving water absorbency for grafting cellulosic water absorbents and resulting in enhanced ultrasonic effects on crystallinity of cellulose.

The degree of cellulose crystallinity depends on the extent of hydrogen bonds [40,43]. Thus, the lower degree in crystallinity of sonicated cellulose compared to that of untreated cellulose means that hydrogen bonds in the cellulose molecules are destroyed by ultrasound waves [5,43]. This claim is based on the fact that ultrasound induced cavitation is able to disjoin the molecules in amorphous and crystalline regions, resulting in destruction of ordered packing of cellulose molecules and the decrease of crystallinity [40,48]. According to the study of Wang et al. [43], when the ultrasonic power increased from 200 W to 700 W, the hydrogen bonds were more thoroughly destroyed effecting a

greater decrease in the degree of crystallinity. Montalbo-Lomboy et al. [49] also reported that switchgrass samples sonicated at 5000 J broke down the chunks of material initially observed into long and small strips of cellulosic material. They concluded that the transition of the material from a very crystalline form of switchgrass into a long strip material is significant and deduced that ultrasonics are able to change the structure of the switchgrass.

3.2.3. Degree of polymerization

Cellulose molecules, as a polymer, have different sizes and weights in proportion with their degree of polymerization (DP), so that larger sizes and weights of cellulose molecules are associated with a higher degree of polymerization [5]. DP is basically related to other cellulose characteristics like crystallinity [10b]. It is believed that glucan chain length plays an effective role in cellulose hydrolysis. Depolymerization of cellulose depends on the nature of the cellulosic substrate. However, regardless of the substrate, it seems that there is a “leveling off” in the cellulose DP, correlated with the increased recalcitrance of the residual crystalline cellulose [1a]. DP is generally determined via viscosity as well as by size exclusion chromatography (SEC) [5,45].

Some researchers concluded that ultrasound irradiation is able to decrease the DP of cellulose [5,45,50]. The cavitation collapse can disrupt the polymeric structure of cellulose to produce lower DP [50b]. The reduction in the degree of polymerization is typical for heterogeneous reactions of cellulose hydrolysis [51]. A higher intensity of ultrasonic treatment can significantly increase degradation of cellulose compared to that of low intensity ultrasonic treatment. Within the same period of time, DP of treated cellulose tends to be smaller as well as its distribution narrower as the intensity of ultrasound waves increases [45,50].

However, Wang et al. [43] reported that ultrasound irradiation alone could change the DP of cellulose very little. Although a high-power ultrasonic treatment (200–500 W for 5–20 min) was used in the study, they believed that the energy of ultrasonic treatments was still low and not large enough to break the chemical bonds of cellulose molecules. The required energy for cleavage of the chemical bonds and hydrogen bonds in cellulose molecules is estimated at about 400–1000 kJ/mol and 5–30 kJ/mol, respectively [5]. The maximal output energy of ultrasonic apparatus adjusted in the study conducted by Wang et al. was 600 kJ, which could only destroy the hydrogen bonds in the cellulose molecules [43]. While the maximal output energy of ultrasonic (600 kJ) was higher than the required energy for cleavage of some chemical bonds, it seems that apart from wasted energy, useful energy through the fluid was less than 400 kJ. However, it is suggested that disruption of the chemical bonds can be enhanced with cooperation of alkali solutions [40,43].

The decomposition of cellulose in nitric acid solutions has also been studied by Gert et al. [52]. Nitric acid is a multifunctional reagent with respect to cellulose. It is illustrated in another study that ultrasonic treatment of cellulose soaked in nitric acid caused a significant decrease in DP of cellulose [51]. A rapid decrease occurred in DP of the initial cellulose at the beginning of interaction of sonicated cellulose with nitric acid, with depolymerization slowing over time. IL-treated cellulose under ultrasonic conditions also displayed a lower molecular weight than by conventional heating. Thus, more disruption can occur in cellulose molecules soaked in ionic liquid media (ILs) by ultrasound irradiation, resulting in lower degree of polymerization [47].

Sun and Tomkinson [53] also reported that as a result of ultrasonic irradiation with a sonicator of 100 W and 20 kHz, degree of polymerization and molecular weight of crude cellulose in wheat straw decreased from 1666 and 269,960 to 1594 mL/g and 258,280 g/mol, respectively, with an increase in ultrasonic

treatment time from 0 to 35 min. However, treatment of 150 mg wheat straw cellulose with 5.0 ml 80% acetic acid (v/v) and 0.5 ml of concentrated nitric acid (70%, v/v) at 120 °C for 15 min, resulted in a sharp decline in the molecular weight (44.65 g/mol). It seems that ultrasound effects on decreasing DP is much lower than acid or alkali treatment, because after acid or alkali treatment, cellulose subjected to ultrasound irradiation showed no significant decrease in its degree of polymerization [43,51,53].

Mason and Lorimer [54] conducted a study on investigation of the hydrodynamic force and shear stress generated from collapse of bubbles, which are the outcome of the ultrasound treatment of polymeric solutions [45]. Tested samples were subjected to ultrasound irradiation for a maximum of 0.5 h. Further reduction in molecular weight of cellulose was related to a prolonged period of sonication. The decrease in molecular weight related to solutions of chitosan [55] and cellulose nitrate [50b] subjected to ultrasound waves for 100 h and 1.5 h, respectively, asymptotically approached a plateau in the degree of polymerization [45].

The overlays of molar mass distributions of cellulose prior to and at various stages of ultrasonic degradation, up to 13 h, are shown in Fig. 9 [56]. The molar mass distribution of cellulose tends to narrow according to increasing exposure of the solution to ultrasound waves. It is worth mentioning that changes in the molar mass distributions occur at molar masses above 100 kg/mol [56].

3.2.4. Morphology

Rougher and cracked surfaces are preferable for the adsorption of enzyme [42,43,45,57]. The studies on the morphological structure of sonicated cellulose assayed by using scanning electron microscope (SEM) and transmission electron microscopy revealed that the dimensions of the cellulose samples had been significantly decreased, especially when samples were subjected to a direct sonication with ultrasound. When materials present in a liquid suspension are exposed to ultrasound, the particles are affected by either surface erosion via cavitation collapse in the surrounding liquid or size reduction due to fission through inter-particle collision or the collapse of bubbles formed on the surface of the materials [35]. After subjecting switchgrass soaked in ammonia to 2000 J of ultrasound irradiation, the size and bulk volume of the material was reduced compared to the unsonicated

samples. However, there were still large pieces of cellulose materials remaining in the sonicated samples at 2000 J, but not at 5000 J [49].

The particle size of microcrystalline cellulose can be significantly reduced under pretreatment by direct ultrasound irradiation [42,58]. Lignocellulosic substrates exposed to ultrasound waves are effectively milled by the shock waves generated by the collapse of bubbles. This assisted in the increase of total surface area of wood sawdust exposed to enzymatic attack.

However, the particle size of rice hull, after pretreatment using the ultrasonic system (250 W, 40 kHz), did not significantly decrease, which could be due to the high ultrasonic frequency used in the study conducted by Yu et al. [35]. According to their observations, ultrasound irradiation was at least able to create surface erosion due to the cavitation collapse in the surrounding liquid. Zhang et al. [40] reported that the surface conformation of the granulated raw material is not changed. An ultrasonic power of 80 W was used in their study, which seems to be low and unable to generate violent cavitation to cause changes to the structure of granules.

The morphology and structure of cellulose fibers have changed after ultrasound treatment less than 400 W and 360 s, so that peeling in the form of large pieces or in the form of smaller flakes and partial fibrillation were observed [57b]. On the contrary, the ultrasound with power of 500 W for 150 s assisting polyethylene glycol (PEG) treatment was not able to reduce the size of cellulose fibers [44]. It seems that PEG was dispersed in the cellulose network with assistance of ultrasound to weaken the original inter- and intra-macromolecular hydrogen bonds without breaking the surface morphology of cellulose, resulting in the relatively lower crystallinity.

Almost all of the related studies reported that ultrasound irradiation treatment could change the surface morphology of cellulosic samples [5]. Erosion was found on the surface of lignocellulosic material [58]. The study of Wood et al. revealed that ultrasound irradiation converted the sample surface into a tangle of filaments, each 20–40 nm in diameter and many microns in length [57a]. It was revealed that many concave pits and cracks will form on the surface of sonicated MCC, which induce an increase of specific area and the improvement of accessibility [5,43]. Tang et al. [57b] also observed the peeling of a sample surface in the form of large pieces or in the form of smaller flakes.

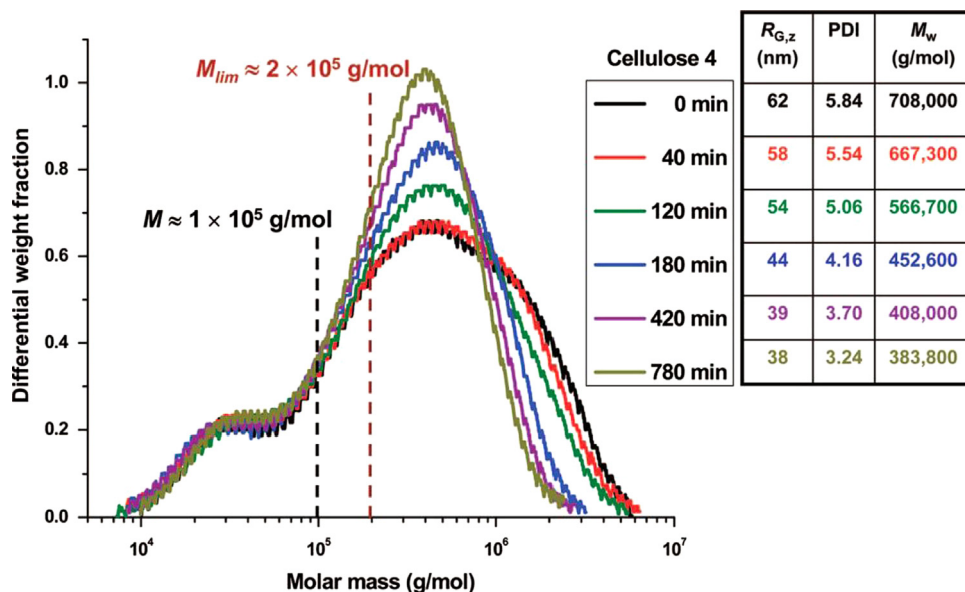


Fig. 9. Overlay of the molar mass distributions of cellulose, at various sonication times (reproduced from Ref. [56]).

Easson et al. [59] reported that ultrasound-induced pitting increases substrate surface area of switchgrass and affects the reaction rate and yield. The structure of sweet sorghum exposed to the ultrasound irradiation was also opened up to resemble more sponge-like structures, which could also provide higher surface areas [46].

The findings of the study performed by Yang et al. [47] indicated that the structures of IL-treated cellulose under ultrasound became even looser and less compact, illustrating the partial disruption of linkages in cellulose. Phosphoric acid treatment with sonication seemed to completely disintegrate the lignocellulosic fibers in bagasse and made a uniform structure with less vacant area [46]. It is worth mentioning that acid pretreatments, however, also produce lignin that obscures the access of cellulose enzymes to cellulose through surface redeposition. The use of ultrasound irradiation before enzyme hydrolysis may disrupt lignin–cellulose hydrophobic interactions and enhances the enzymatic conversion of cellulose into fermentable sugars [11].

3.3. Effect of ultrasound on sugar yield

Because sugar and ethanol yields are usually considered proportional, many studies were conducted only for sugar yield [5]. Lignocellulose materials treated with ultrasound irradiation could obtain higher sugar yield following hydrolysis compared to that without ultrasound [39,57a]. Yachmenev et al. [26a] reported that ultrasonic treatment could increase the sugar yield by more than 10%. Rezaei et al. [60] found that the amount of released glucose for sawdust slurries sonicated at a frequency of 20 kHz and a power output of 120 W for 2 h, 4 h and 12 h was found to be 22%, 43% and 53% higher compared to that of the unsonicated sample, respectively. Lignocellulosic materials irradiated with ultrasound before enzyme hydrolysis may disrupt lignin–cellulose hydrophobic interactions and increase the enzymatic conversion of cellulose into fermentable sugars [11]. Ultrasound irradiation was also reported to be extremely effective in delignifying wheat straw, so that the residual lignin content for pulp and paper production was reduced by more than 75% [61]. Corn slurry samples exposed to ultrasound also released 30% more sugar than untreated samples [62]. Ultrasonic treatment together with aeration provided much higher efficiency in hydrolysis of cellulosic materials. The hydrolysis rates for samples of sonicated with and without aeration were determined to be 0.28 ppm/min and 0.07 ppm/min glucose, respectively [63].

Low level uniform ultrasound waves applied to the cellulosic solutions containing enzyme do not reduce the specific activity of enzyme macromolecules in any significant way [26a]. Recent investigations revealed that the use of proper intensity ultrasound waves can enhance the permeability of cell membranes [40,55] and improve the catalytic activity of enzymes [40,64]. Using the ultrasound waves in the reaction solutions related to the enzymatic hydrolysis of corn stover and sugar cane bagasse samples caused a significant improvement in enzyme efficiency [26a]. The ultrasound acts directly on the cellulosic substrates and cause them to partially degrade, leading for easier enzymatic reaction. Micro-jets and shock waves generated by ultrasound cause a certain degree of impact to the enzyme molecules together with the substrate, resulting in increasing their contact time and subsequently increasing the reaction rate with a faster release rate of sugars [40].

NaOH treatment together with ultrasound irradiation was found to be an efficient alternative for pretreatment of bagasse before enzymatic hydrolysis [46]. Alkali treatment combined with ultrasound is capable of reducing the reaction process intensity, the reaction time and the amounts of alkali required [40]. In comparing pretreatments of NaOH and phosphoric acid with and without ultrasound, the hydrolysis of pretreated bagasse by NaOH

solution assisted with ultrasound was the most effective treatment, where more than 92% of the theoretical glucose yield was obtained within 72 h [46]. The decomposition of the alkali treated substrate subjected to ultrasound irradiation can be higher than that of the alkali pretreatment alone [40]. Although early during hydrolysis, ultrasonic-assisted NaOH significantly enhanced the hydrolysis rate, NaOH pretreatment alone finally achieved the same glucose yield as the ultrasonic-assisted NaOH treatment [46]. However, Zhang et al. [40] reported that by using ultrasound waves, the same decomposition rate of the raw material can be obtained if the alkali concentration is reduced from 8% to 5%, as well as the hydrolysis time is reduced from 1.5 h to 30 min. This improvement can be due to the mass transfer intensification and the alkali solubility enhanced by the cavitation effects introduced by the ultrasound [5,42]. The collapse of bubbles generates the energy, which is transmitted to the hydrolysis process resulting in improving the process of mass transfer and diffusion of the enzyme to the substrate and the product entering the solute. Ultrasound waves can also change the conformation of the enzyme molecule, which causes it to assume a more suitable structure for combination with the substrate, so that the ultrasound waves are found to exert a catalytic function [40].

Montalbo-Lomboy et al. [49] reported that switchgrass treated by ultrasonic-assisted ammonia released about 10% more fermentable sugars compared to that of ammonia treatment alone. In another study, it was found that the ultrasonic treatment of switchgrass resulted in an increase of 7.5% in releasing fermentable sugars, but 9.3% in experiments using ultrasound on switchgrass soaked in ammonium hydroxide [59]. However, the net energy balance was not favorable and further investigations on re-evaluating the design and conditions were recommended in order to improve ultrasonic treatment of lignocellulosic materials.

The synergistic effect of ultrasound and acid catalyzed reaction can considerably reduce the time required for hydrolysis [46,65]. This improvement can be due to decreasing the inhibitors observed in ultrasonic-assisted acid hydrolysis [65]. Aguilar et al. [66] reported that hydrolysis with 2% H₂SO₄ at a temperature of 121 °C was found to generate 3.65 g/L of furfural, whereas in another study conducted by Velmurugan and Muthukumar [65], the furfural concentration observed was very low (0.1 g/L) in the hydrolysis with 2% H₂SO₄ subjected to ultrasound due to the lower reaction temperature possible.

Combining ultrasound and ILs has proven to be an effective pretreatment of lignocellulosic materials [32,47,67]. The cellulose saccharification ratio of kenaf powder in EmimOAc was 86% after only 15 min of ultrasonic pretreatment at 25 °C, compared to only 47% in the case of thermal pretreatment at 110 °C for 120 min in the study by Ninomiya et al. [32]. In another study, cellulose dissolved in ionic liquids with ultrasonic heating pretreatment was found to increase conversion to glucose by 53% compared to that of untreated cellulose. Similar research has demonstrated that ultrasound pretreatment can enhance the dissolution of cellulose in ionic liquids [67]. The cellulose saccharification by pretreating lignocellulosic in EmimOAc can reach 90–95%, even when the lignin content in the pretreated lignocellulosic material was 60–70% of that in the untreated original samples [32,68]. These findings mean that it is not necessary to remove all lignin present in the lignocellulose. Rather, the disruption of the lignin structure is enough that the enzyme molecules can access the polysaccharides to achieve an almost 100% cellulose hydrolysis into glucose [32]. The ionic liquid pretreatment together with ultrasound waves might be key to the disruption of rigid lignin structures. However, further investigations into the synergetic effects of ultrasound and ionic liquids on the physical and chemical properties of lignin is recommended.

Table 5

Simplified kinetic model related to enzymatic hydrolysis of cellulose [39,69].



$$\text{Rate of production of total sugar } \frac{dC}{dt} = \frac{kE_0(C_\infty - C)}{K_M[1 + (1/K_I)C] + 0.9(C_\infty - C)} \quad (6)$$

$$\text{Equilibrium constant } K = G/O = G/(C - G) \quad (7)$$

Where EG, CBH and BG are endoglucanase, cellobiohydrolase and b-glucosidase, respectively; C_∞ , k , K_M and K_I are ultimate total sugar concentration, apparent rate constant, apparent Michaelis constant and apparent inhibition constant, respectively, and $C = O + G$

Table 6

Summary of various processes used for the pretreatment of lignocellulosic biomass [1a].

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	The energy consumption as a function of final particle size and comminution ratio, often higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
Ammonia fiber explosion	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	Not efficient for biomass with high lignin content
CO ₂ explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash/char production
Pulsed electrical field	Ambient conditions; disrupts plant cells; simple equipment	Process needs more research
Biological	Degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low
Ultrasonic	Increases accessible surface area; disrupts plant cells; does not produce inhibitors for downstream processes	Process needs more research

A novel two-step pretreatment for enzymatic hydrolysis of rice hull (RH), including a mild physical step by ultrasound and a subsequent biological treatment by *Pleurotus ostreatus* was introduced by Yu et al. [35]. After a combined pretreatment of ultrasound (250 W, 30 min) and *P. ostreatus*, the net sugar yield obtained was 32.2%, which was about 4.2 times higher than that by fungal pretreatment alone. The combined pretreatments notably enhanced the delignification rates and reduced the losses of the carbohydrates, which effectively shortened the residence time.

Although many studies during the last decade in the field of ultrasonic treatment have focused on the aspect of microbial reactions [39], a few kinetic studies have been conducted on the effects of ultrasonic intensity and reactor size on the enzymatic reaction [69]. An ultrasonic power of 30 W applied to waste paper solution in scaling up from 0.8 L to 3.2 L was able to significantly enhance the saccharification although it was less efficient in scaling up from 3.2 L to 6.4 L. This phenomenon might be attributed to the decrease in specific ultrasonic intensity with scale-up. It is interesting to note that the heat generated by ultrasound irradiation is more easily controlled in larger scale reactors. Simplified kinetic models for enzymatic hydrolysis of cellulose in different scale ultrasonic reactors are presented in Table 5, which are explained in detail in the literature [39,69]. The apparent ultimate conversion of cellulosic substrates can be correlated as a function of the ratio of the initial substrate to enzyme concentration in each fixed specific ultrasonic intensity

range. Another result related to the design of ultrasonic reactors was that periodic application of ultrasound for 120 min with 15 s on and 105 s off could generate about 20% higher sugar yield than continuous ultrasound [5,42].

3.4. Effect of ultrasound on ethanol yield

Wood et al. [57a] reported that ultrasonic treatment could generate a nearly 20% ethanol enhancement from mixed waste office paper after 96 h of fermentation. The ethanol yield was directly correlated with the ultrasonic treatment time. It was also observed in their study that periodic ultrasound irradiation for 120 min with 15 s on and 105 s off could increase ethanol yield about 30% compared to that of continuous ultrasonic. Ultrasound-assisted NaOH also enhanced ethanol yield from sweet sorghum bagasse by the fungus *Mucor hiemalis* by up to 81%, which was the best result for ethanol production among all the applied pretreatment techniques such as NaOH and phosphoric acid with and without ultrasound in the study of Goshadrou et al. [46]. Velmurugan and Muthukumar [65] conducted a study on the production of sugar monomers from sugarcane bagasse (SCB) by sono-assisted acid hydrolysis to analyze the potential fermentability of produced sugars using *Saccharomyces cerevisiae*. They reported an ethanol yield after 60 h of incubation of 8.11 g/L, which corresponds to 91.8% of the theoretical yield. Cheng et al. [70] obtained about 0.36 g/g as the ethanol yield of sugars during the fermentation of hydrolyzed SCB. In the study performed by Velmurugan and Muthukumar, the

ethanol production observed was 0.43 g/g of glucose. *S. cerevisiae* did not utilize pentoses released during ultrasonic-assisted acid hydrolysis [65,71].

Generally, there are both advantages and disadvantages of ultrasonic pretreatment of lignocellulosic biomass compared to other pretreatments as explained by Kumar et al. [1a]. In order to complement the previous work, the properties of ultrasonic pretreatment have been compared in summary with various other pretreatment processes in Table 6. It must be emphasized that it is not always possible to transfer the results of pretreatment from one type of biomass material to another. The choice of a pretreatment technology used for a particular biomass is dependent on the composition and the byproducts produced as a result of pretreatment. These factors significantly affect the costs associated with a pretreatment method.

4. Starch

4.1. Process of producing ethanol from starch

4.1.1. Structure

Starch granules are semi-crystalline particles in a variety of size from 1 μm to 100 μm and structure dependent on botanic origin [72]. The basic building block of these semi-crystalline particles is a glycosyl monomer in size of around 0.3 nm. In general, the structure of semi-crystalline native starch granules is found as a hierarchical structural periodicity (Fig. 10), having a layered organization with alternating radial rings of amorphous and semi-crystalline material in thickness of 120–400 nm [73]. Although the amorphous rings are formed by amylose and amylopectin in a disordered conformation, the semi-crystalline rings consist of a lamellar structure of alternating crystalline and amorphous regions with a regular repeat distance of 9–10 nm [9].

Amylose is a linear polymer connected by α -1,4 glycosidic bonds and amylopectin is assumed highly branched connected by both α -1,6 and α -1,4 glycosidic bonds [72]. Amylopectin clusters involve amylose molecules that pass through both the crystalline and amorphous layers. The amylose molecules present in crystalline regions are considered to be in a straightened conformation, whereas these molecules are found in a disordered conformation in amorphous regions [9].

4.1.2. Conversion of starch to ethanol

Rupture and disintegration of the compact crystalline granular structure in water through a process known as gelatinization is an important mechanism in starch processing [74]. Indeed, the

process of starch gelatinization is the loss of the semi-crystalline structure or the melting of starch crystallites in the presence of water, which is dependent on other factors such as temperature and solid-to-liquid ratio [6]. The starch granules, during gelatinization, swell and exude part of the amylose, leading to more susceptibility to enzyme degradation [75]. Enzymes are considered an important factor in the breakdown of starch. Alpha-amylase is known to act on both solutions of starch granules and insoluble starch. Glucose can be produced from the action of amyloglucosidase on α -amylase degradation products [6]. The liquefied starch as crude mash can simultaneously be saccharified and fermented in one vessel. By adding amyloglucosidase enzyme and yeasts to the slurry concomitantly, a process of simultaneous saccharification and fermentation (SSF) might be conducted in a single reactor [6].

4.2. Effect of ultrasound on physical characteristics of starch

4.2.1. Swelling power and disintegration

When starch granules are heated in water, they swell and their size and shape changes. Blocklet-like structures scatter, crystalline regions are irreversibly disrupted, birefringence is lost as double helices unwind and starch becomes soluble [76]. This molecular disordering is known as gelatinization, and is initiated by swelling of amorphous growth rings within the granule accompanied by leaching of amylose from the granule [9]. Disruption of starch crystallites can occur after the amorphous regions absorb a significant amount of water, which provides a sufficient stress through connectivity of molecules from the amorphous region to the semi-crystalline lamellae. In general, the structural changes of starch during gelatinization include a crystallite melting and double-helix unwinding, absorption of water in the amorphous region of starch, as well as displacement of amylopectin units and leaching of amylose from the granules [9,76]. The ultrasonic treatment enhances disintegration of starch granules, leading to an acceleration of starch hydrolysis due to exposing a much larger surface area to enzymes, increasing the release of fermentable sugars and thereby increasing ethanol productivity [62,77]. Nearly complete disintegration of corn starch and cassava cells was observed with large numbers of fragmented cell materials by high-power ultrasonic treatment for 40 s [11,62,78].

The swelling power of sugary corn (containing 30% amylose) subjected to ultrasound irradiation was initiated as quickly as 5 s and increased rapidly [79]. The swelling power and the rate of degradation related to ultrasonicated samples are accelerated at or above the gelatinization temperature [80]. The rate of swelling

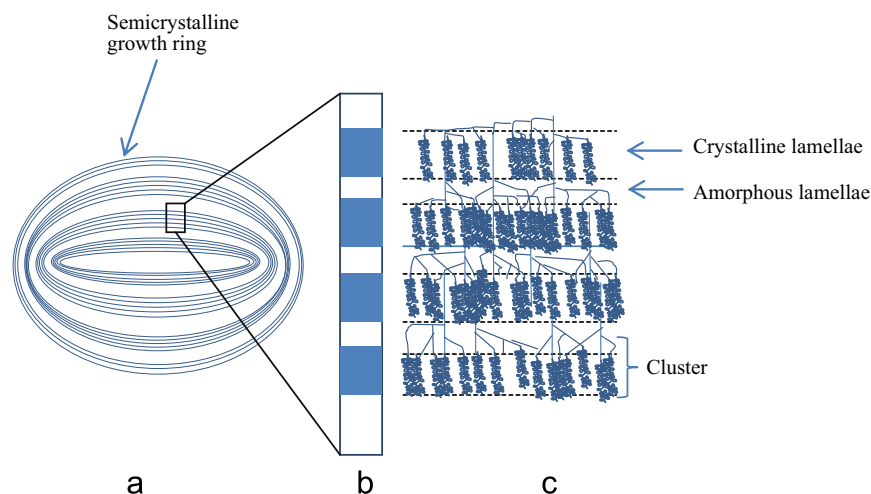


Fig. 10. Overview of starch granule structure: (a) whole granule, (b) lamellae, and (c) polymer chains (adapted from Ref. [73]).

Table 7

The degree of granule disintegration and the degree of solubilization of starches and polysaccharides at 65 °C, 75 °C and 85 °C [82].

Samples	Sonication time (min)	Degree of granule disintegration			Degree of solubilization		
		65 °C	75 °C	85 °C	65 °C	75 °C	85 °C
Waxy maize	0	3.5	25.7	22.6	0.00	0.28	0.48
	1	2.9	0.6	0.5	0.11	0.96	0.94
	5	2.5	0.5	0.4	0.16	0.99	0.95
Potato	0	28.8	104.5	110.2	0.08	0.00	0.00
	1	2.9	1.8	2.3	0.63	0.89	0.90
	5	1.1	0.5	1.2	0.71	0.93	0.94
Tapioca	0	22.0	26.9	31.0	0.13	0.17	0.24
	1	1.7	0.8	0.8	0.80	0.96	0.96
	5	0.6	0.5	0.8	0.84	0.99	1.02
Sweet potato	0	2.5	6.5	19.7	0.01	0.05	0.15
	1	2.1	2.4	0.5	0.02	0.38	0.91
	5	2.0	1.7	0.3	0.03	0.43	0.90
Corn	0	4.4	9.1	9.5	0.02	0.05	0.08
	1	4.7	6.4	7.7	0.02	0.09	0.22
	5	4.2	4.8	3.1	0.03	0.45	0.71

(gelatinization) is higher with ultrasonic treatment in comparison to that of heating only. Montalbo-Lomboy et al. [79] reported that the final swelling power of sugary corn is substantially higher (around 6 g/g) for the ultrasonic treatment compared to samples that were heated only (2.8 g/g). This increase in swelling power is believed to be due to the fact that ultrasonic treatment affects the amorphous regions of starch and enhances water absorption [64]. Aside from that, ultrasound waves can gradually separate starch agglomerates and reduce starch molecule sizes, which promotes the solubilization of the amylase in the suspension into a three-dimensional gel network [81]. The degree of granule disintegration increases as the swelling of the starch granules treated by heat proceeds as the temperature increases (Table 7). With the ultrasonic treatment as short as 1 min, the swelled granule disintegrates and a certain amount of the contents is solubilized [82]. With increasing ultrasound power and intensity, the swelling power increases. The increase in swelling power of starches subjected to ultrasound irradiation is also higher for samples at higher temperatures [83].

The swelling power of sugary corn-2 (from 2.5 g/g to 5.0 g/g) was found to be higher than that of commodity corn (1.9–2.6 g/g) in the varied conditions of sonication [79]. In contrast, some studies considered that swelling power is lower for sugary corn starches in comparison with normal corn starches [84]. This might be explained by the fact that Montalbo-Lomboy et al. [79] used ground corn instead of corn starch in their study. It should be noted that starch used in the study conducted by Montalbo-Lomboy et al. is embedded in the corn kernel matrix, which may be more difficult to gelatinize than free granular starch. However, ultrasonic treatment can enhance the release of the starch.

4.2.2. Particle size

Most of effective factors in the conversion of starch to ethanol such as starch composition, solubility, gelatinization and pasting properties, enzyme susceptibility, crystallinity, and swelling are all affected by the granule size [34]. The reduction in particle size and opening up of starch fibrous structure can decrease the dose of enzymes needed, shorten the conversion time of starch to ethanol, improve the efficiency of the starch hydrolysis, increase the overall sugar yield, and even eliminate some of the unit processes, which ultimately results in an overall improvement in ethanol yield and reduction in cost [78]. Reduction in starch particle size can also enhance mass transfer and lead to an increase in enzyme activity [62].

Several studies have investigated the effects of ultrasound on the particle sizes of starches [62,77,78,83,85]. The hydrodynamic shear force introduced by ultrasound irradiation causes the disintegration of starch particles into a slurry into finer particles, significantly enhancing the surface area for enzyme activity [85a]. A reduction of 10–20-fold in corn particle sizes derived from a dry-grind ethanol plant following ultrasound pretreatment was observed by Khanal et al. [62]. They reported that ultrasound pretreatment enhanced glucose release during enzymatic hydrolysis of corn meal mainly due to reduction in particle size. Reduction in particle size causes better mixing and the release of starch that was bound to lipids and did not have access to the hydrolyzing enzyme [77]. In other studies a 20-fold reduction in corn particle size following ultrasonic treatment was reported [62]. Particle size reduction of rice grains resulted from ultrasonic treatment caused in shorter cooking times and faster gelatinization [86]. Ultrasonic treatment reduced cassava particle size nearly 40-fold at an output power as high as 8 W/mL [78]. The ultrasonic treatment not only contributes to the particle size reduction of starches in solutions, but also heat generated assists starch gelatinization, which is required in enzymatic starch hydrolysis [85a]. Luo et al. [85b] reported that ultrasonic treatment did not alter the size and shape of the maize starch granules that were not dissolved in water as cavitation only forms in the liquid phase. However, the ultrasound could develop some porosity on the surface of normal and waxy maize starches. Microwaves affected granule degradation of corn meal after liquefaction even more than ultrasound. However, both pretreatments were very effective in enhancing the enzymatic hydrolysis in comparison with conventional heating [77,87].

The volume weighted mean particle size is inversely proportional to the applied sono-energy densities [86]. Particle size reduction is directly proportional to power level and sonication time. The particle size reduction of starches at the higher power level and longer sonication time enhance glucose yield under similar conditions. The relative volume of corn slurry increased as the ultrasound intensity level increased for both 20 s and 40 s of sonication. These data are in close agreement with Khanal et al. [62], where starch size was inversely proportional to ultrasound intensity. The extent of damage to starch granules suspensions is only slightly proportional to the ultrasonic intensity at the beginning, then increases markedly with the ultrasonic intensity after a power threshold is reached [26b]. Jambrak et al. [83] observed that when applying more than the optimum ultrasonic intensity, starch granules tended to agglomerate becoming larger in shape and size, which is attributed to liberated bonds that offer the opportunity to connect linkages between polymers. However ultrasound with an optimal intensity can reduce the starch granule size and consequently change the physical/chemical properties of starch.

Particle size distribution of sonicated starch is not absolutely lognormal. In the results reported by Montalbo-Lomboy et al. [86], at 50% probability the particle size of control (without sonication) was 361.8 µm while particle sizes of low (140–153 W), medium (214–228 W) and high (199–298W) ultrasonic power were 115.2 µm, 61.0 µm and 35.2 µm, respectively. Montalbo-Lomboy et al. also reported that the particle size reduction related to corn slurry samples treated in a batch ultrasonic system was more than that of the samples treated in continuous-flow ultrasonic system (Fig. 11). The higher ultrasound intensity in a batch system enhanced the reduction of particle size compared to that of the lower energy input applied to samples in continuous flow systems. In their experiment, the energy densities of the batch and continuous ultrasonic systems ranged from 87 kJ/L to 260 kJ/L and 4–14 kJ/L, respectively. However, due to the large volume involved in full scale processes, they recommended a continuous ultrasonic system in order to pretreat starch for its conversion to

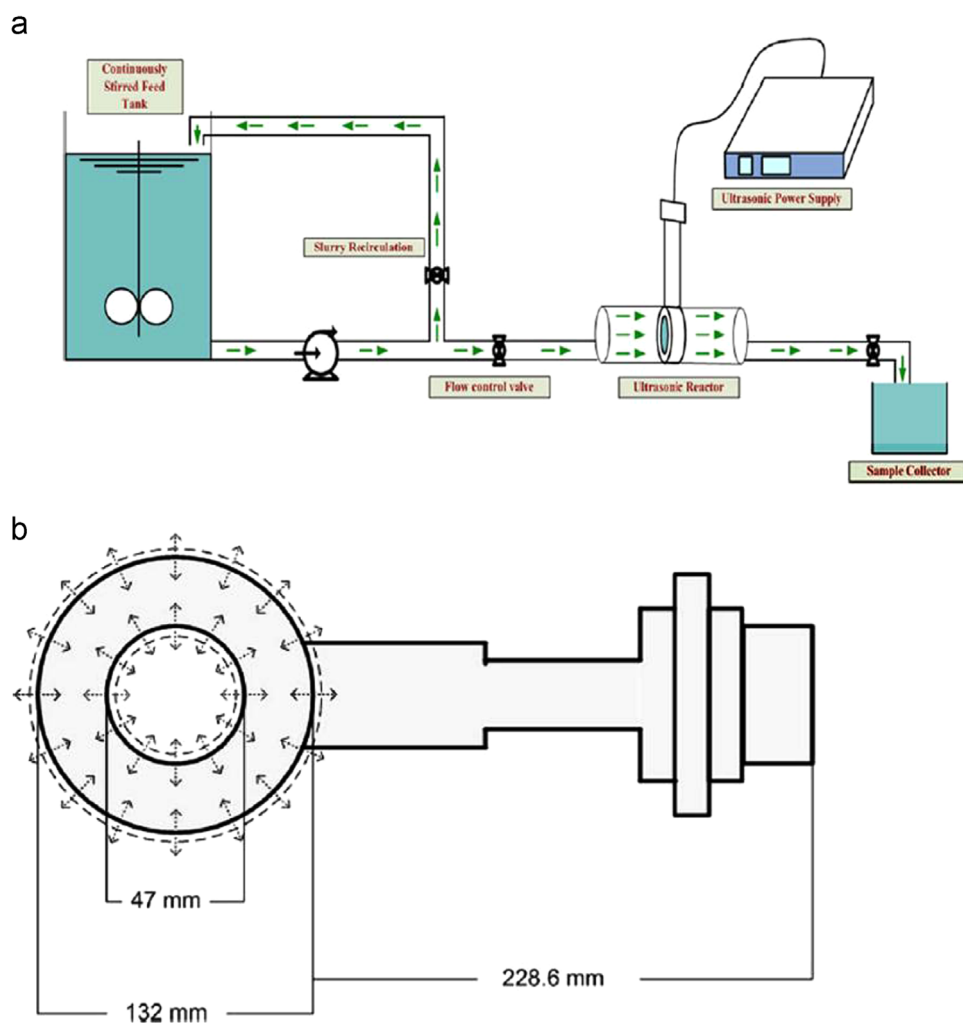


Fig. 11. Schematic of (a) continuous flow experimental set-up and (b) Branson ultrasonic "donut" shaped horn, used in the experiments of Montalbo-Lomboy et al. [86].

ethanol. Reduction in the particle sizes of starches subjected to ultrasound probes is also markedly more than that with ultrasound bath [83].

According to Khanal et al. [62] the peak of particle size distribution curve shifted from 800 μm to around 80 μm following sonication at high power levels (475 W, 40 s) for cooked corn slurry samples. In another study, the inflection point of the particle size distribution curve of corn slurry was shifted from 500 μm to approximately 20 μm following sonication (199–298 W, 40 s) [86]. Three peaks of 600 μm , 200 μm , and 15 μm were found in the particle size distribution curves of cassava chip slurry, while the peak of 600 μm was shifted to 200 μm and 15 μm following ultrasonic treatment especially at medium and high power levels. Although the number of 600 μm particles decreased after sonication, this peak is expected to represent milled cassava particles that were not affected by ultrasound treatment. In contrast, the 200 μm peak was related to cell morphologies of the cassava chips affected by sonication. The 15 μm peak corresponded to the individual starch granules [85a]. Such a decrease in the particle size distribution due to ultrasound is in agreement with the results reported for corn slurry [62], for uranium ore slurry [88] and for sorghum slurry [89].

4.2.3. Morphology

Ultrasonic pretreatment can alter the structure of starches [11,77,87]. The destruction of starch structures might result in release of more individual starch granules to the aqueous phase,

which enhances the enzymatic hydrolysis [78]. Structural changes of starch granules pretreated after the liquefaction is started were more than that before liquefaction. However, ultrasound is more effective in decomposing starch in comparison with conventional heating [77,87]. Ultrasound during just 20 s pretreatment could partially rupture the starch granules. When inspected under high magnification, the granules were coated with what is believed to be gelatinized starch. Starch granules might be more gelatinized with increasing treatment time by using ultrasound, so that after 40 s the samples appeared to be fully gelatinized [62,79].

The final temperature in the reaction chambers increases in direct proportion to the power setting and the treatment time, because ultrasound waves in the reactions can produce heat, which can be effective for breaking down intra-molecular bonds and improving the starch gelatinization process [79]. Under temperature-controlled conditions, the disintegration of cassava cell structures was directly proportional to power and time of sonication so that near-complete disintegration of cassava cell structures was observed in samples subjected to ultrasonic power of 8 W/mL for 40 s [78]. Similar results were reported for other types of starches [90].

Fig. 12 shows polarized microscope images of sugary starch (A) without and (B) with pretreatment [79]. The untreated samples display a clear Maltese cross pattern, which is typical for ungelatinized starch granules [91]. These spherulites are related to semi-crystalline structures of starch. As can be seen from the figure, spherulites and birefringence were more

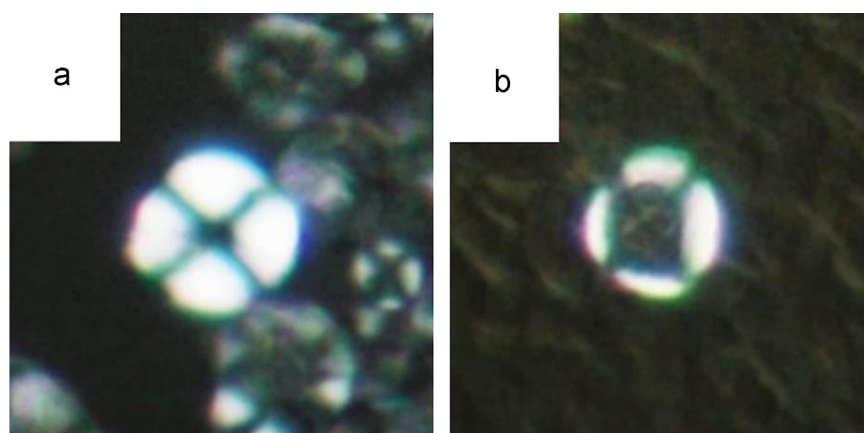


Fig. 12. Polarized microscope images of (A) untreated and (B) sonicated samples shown at 22.5 magnification to emphasize the Maltese cross pattern in the starch granule (reproduced from Ref. [79]).

pronounced in the untreated starch samples (Fig. 12A) compared to the sonicated samples (Fig. 12B). It is also known that the amount of birefringence is proportional to the degree of crystallinity [91]. The starch samples pretreated by ultrasound had no birefringence in the center of the spherulite [92], which indicates that the samples had a lower degree of crystallinity and were partially gelatinized. In other research, the starch granules and their birefringence were not observed in the cooked slurry samples both with and without ultrasonic pretreatment. Heat treatment not only played a positive role in improvement of starch gelatinization (loss of birefringence) but also destroyed the starch granule structures. However, starch granules disintegrated by ultrasound irradiation generated more ethanol than only heat treated starch granules [85a].

Huang et al. [64] reported that many Maltese crosses do not disappear after ultrasound treatment of corn starch granules. They believed that ultrasonic treatment cannot destroy the whole structure of the starch granules but may change parts of them and the holes formed on the surface of the sonicated starch granules are likely the amorphous parts. Many micropores with different sizes were observed on the surface of ultrasound-treated normal and waxy maize particles. The pores are generally evenly distributed and take on alveolar shapes in some granules [62,64,85b]. Internal cavities at the granule hilum, together with formed pores at the surfaces of starch granules sonicated as well as channels connecting the two can enhance the efficiency of granule reactions [93]. Thus, the holes may increase the surface area of the starch and the channels may permit reagents to more easily penetrate into the bulk of the granule, which may enhance the speed of chemical reactions [64].

4.2.4. Depolymerization

Degree of polymerization (DP) of amylose and amylopectin are considered in the range of 50–6000 and 30,000–300,000 glucose units, respectively [94]. A strand of amylose is reported to have molecular weight in the range of 104–106 Da and estimated around 107–108 Da for an amylopectin strand [6].

Ultrasonic treatment for depolymerization has been considered on a variety of homo- and hetero-polysaccharides such as dextran [95], pullulan [96], chitosan [97], hyaluronic acid [98], xyloglucan [99] and starch [100]. Ultrasound irradiation can cause rupturing and mechanical damage in the starch granules by collapse of bubbles, which in turn generates the shear forces capable in breaking the polymers chains. Some water present in the solution is also decomposed into OH and H radicals in the collapse of cavitation bubbles. Interestingly, in the process of cavitation, some

of these radicals diffuse out of the bubbles to the surrounding liquid, and then react with molecules present into the solution causing polymer degradation [83]. The shear stress created by stable cavitation is considered effective if able to degrade high molecular weight polymers even without the presence of bubble collapse [101]. Ultrasonic treatment apparently expedites the polymer degradation of starch by separating the amylopectin chains and leaching out amylose chains from the collective entity [82]. The shear forces introduced by ultrasound waves can weaken the interactions between the polymer-molecules and as a result, the viscosity is reduced by the destruction of the polymer network [82].

Seguchi et al. [102] reported that ultrasonic treatment gradually separated starch agglomerate and reduced starch molecular sizes. Isono et al. [80] also observed a reduction in the average molecular weight of waxy rice starch subjected to ultrasound waves. Similar results were obtained by other scientists [26b,79,85b,86]. Vodeníčarová et al. [99] concluded that ultrasonic treatment was the most convenient procedure to decrease the molecular mass in comparison of γ -radiation and microwave treatments. Their results revealed that depolymerization by ultrasound generated products without significant alteration of the primary structure of the polysaccharide, while degradation by microwave treatment altered the composition of sugar due to cleavage of glycosyl side chains. The γ -radiation treatment also generated high-molecular mass components induced by chain cleavage of the polysaccharide during treatment.

According to Iida et al. [82], the molecular weight of waxy maize starch sharply decreased in the initial period of 10–30 min sonication and thereafter the depolymerization continued slowly. Also, the starch molecule chains regularly decrease with increasing ultrasound intensity and progressively approach the minimum chain length [83].

4.2.5. Viscosity

The high mash viscosity causes a restriction in enzymatic activity and having greater reaction time is a way of overcoming this limitation. Increasing the reaction time, however, provides more opportunity for deleterious retrogradation, forming amylose-lipid complexes and an increase in the degree of insoluble sugars [6,103]. Fig. 13 shows a typical profile of pasting viscosity of starches analyzed based on a Rapid ViscoAnalyzer (RVA). As can be seen from the figure, pasting temperature corresponds to the point when the temperature rises above the gelatinization temperature, inducing an increase in both starch granule swelling and viscosity. The peak viscosity represents the

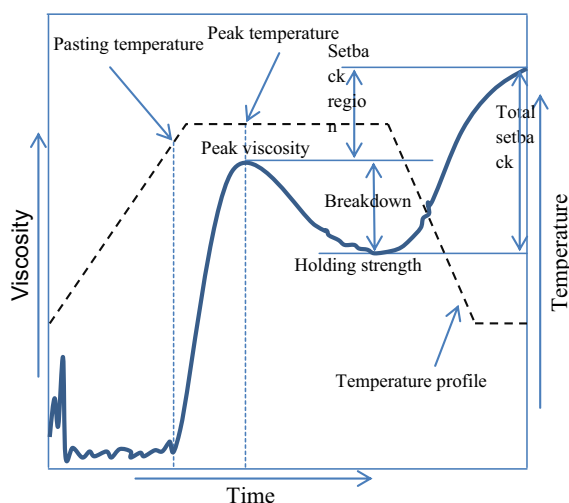


Fig. 13. A typical Rapid ViscoAnalyzer (RVA) pasting profile showing the commonly measured parameters (adapted from Ref. [104]).

maximum viscosity reached during the heating and holding cycle and is illustrative of the water holding capacity of starch [104]. The peak temperature occurs at peak viscosity. The breakdown viscosity is basically proportional to the degree of the disintegration of starch granules as they are heated [105] due to granules rupturing and soluble amylose leaching out. The degree of RVA breakdown is dependent on the solubility of the starch, so that the more soluble the starch, the more it will thin on shearing [6]. As the mixture is cooled, a gel forms due to re-association between starch molecules, especially amylose, and viscosity subsequently increases. Finally, the total setback (indicated region in Fig. 13) involves retrogradation, or re-ordering of the starch molecules. However, it is well known that pasting properties of starch are affected by amylose and lipid contents present in the starch, as well as by branch chain-length distribution of amylopectin [6,104].

According to the pioneering work by Szent-Györgyi [106], ultrasonic treatment can reduce the viscosity of the starch solution. Ultrasound irradiation can cause the physical degradation of starch granules, reduction in starch suspension viscosity as well as a decrease in molecular sizes of starch polymer [85b,90b,107]. A drastic decrease was observed in viscosity of the solutions with 5% and 10% of waxy maize, potato, tapioca, sweet potato, glucomannan (1%) and pectin that were sonicated for 30 min after gelatinization (Table 8) [82]. This viscosity reduction in the sonicated starch solutions was also observed even by cooling to room temperature. In a corn wet milling process, ultrasound irradiation applied to solutions was capable of rapidly removing corn pericarp prior to steeping, resulting in a reduction in steeping time and improvement of the isolated starch gelatinization and pasting properties [101]. However, it seems that high concentration of starch slurries, i.e. 25–30% is a limitation for the depression of viscosity, and might not be effectively affected by sonication due to the formation of firm gel within the concentration range [108]. As a recent finding, the pasting temperature of corn starch subjected to ultrasound irradiation increased while a lower maximum viscosity was observed in comparison to non-sonicated corn starch paste [26b]. Ultrasonic treatment of normal and amylo-maize V starches could result a reduction in peak viscosity, hot paste viscosity, hot paste viscosity after 30 min holding and cold paste viscosity, but the pasting temperature was unchanged [85b]. As a comparison, the extent of the decrease in viscosity by ultrasonic treatment followed the order: waxy maize < normal maize < amylo-maize V. This order is inversely proportional to amylopectin fraction into starch structure. OH radicals generated

Table 8

Changes in the viscosity of starches and polysaccharides by sonication at output power of 100 W. [82].

Samples		Viscosity (mPa s)				
		60 °C	50 °C	40 °C	30 °C	20 °C
Waxy maize	Untreated	254	300	372	440	586
	Treated	8	6	8	10	14
Potato	Untreated	528	782	2100	3110	5410
	Treated	8	8	10	16	20
Tapioca	Untreated	574	900	1420	2110	3200
	Treated	6	8	10	14	16
Sweet potato	Untreated	656	760	912	1140	1310
	Treated	4	6	6	8	12
Glucomannan (1%)	Untreated	3480	4480	4400	6520	7600
	Treated	64	78	104	178	250
Pectine	Untreated	230	206	480	742	1110
	Treated	134	190	296	424	700

The concentrations of slurries were 5% except for glucomannan (1%).

by cavitation might react with linear amylose and side chains of amylopectin. It appears that linear amylose is more easily attacked by ultrasound than highly branched amylopectin [109].

The viscosity of starch solutions subjected to ultrasound irradiation decreases with increasing temperature. This trend might be attributed to the fact that mechanical effects of ultrasound effectively develop in relatively low viscosity conditions, which are associated with higher temperature [82]. Because the cavitation effects at lower temperatures are more effective than the mechanical effects at higher temperature on the viscosity depression of starch solutions, the decrease in viscosity due to rising sonication temperatures is not so remarkable. Glucoamylase catalysis combined with ultrasonic treatment enhanced the combining-water capacity of microporous starch and was strongly effective in breaking starch granules and increasing viscosity [107]. Chan et al. [110] reported that sodium dodecyl sulphate combined with ultrasonic treatment increased the peak viscosity and reduced the pasting temperature for corn, sago and mung bean starches but not potato starch.

As ultrasonic intensity increases, the molecules absorb ultrasound energy and gradually lose their hydration resulting in the lowering of starch viscosity. The consistency coefficient (a measure of the resistance of a fluid which is being deformed by either shear stress or tensile stress) also decreases stepwise jointly with increasing ultrasound intensity [83]. Benmoussa and Hamaker [90b] reported that higher ultrasonic intensity on solutions of potato starch caused more disintegration of starch granules as well as modification of physicochemical properties, as a result, the starch paste became more transparent with significantly decreased paste viscosity at 70 °C. The viscosity of starch solutions might decrease at lower ultrasonic frequencies, around 20 kHz, in comparison with that of higher frequencies [82].

With increasing degree of hydrolysis (DH), the viscosity of starch solutions subjected to ultrasound irradiation decreases, while the pasting temperature as well as temperature of peak viscosity increases [64]. This trend is attributed to the hydrolysis of the amorphous lamellae, resulting in destabilizing the crystalline lamellae by increasing the hydration and swelling of the crystallites. When the structure of amorphous lamellae is degraded, the pasting temperature might increase up to a certain degree of DH, afterwards as the DH increases, the onset and peak temperatures of viscosity decrease, likely due to degradation of crystalline lamellae [111]. The enthalpy of gelatinization (ΔH) is primarily proportional to the loss of double-helical order with unraveling and melting during gelatinization. At low DH, ΔH values of starch solutions treated by ultrasound might be enhanced because the

amorphous regions are degraded prior to the crystalline regions. However, some of the double helices present in crystalline and in non-crystalline regions of the starch structure will be disrupted at higher DH, which results in a decrease in ΔH values of the starch [26b,64].

4.3. Effect of ultrasound on hydrolysis

Ultrasound irradiation during starch hydrolysis was used as early as 1933 by Szent-Gyorgyi [106]. Ultrasound waves can generate hydrodynamic shear forces in the aqueous starch solutions, which assist the disruption of coarse particles and fibers of starch present in the solution [85a]. The destruction of starch cell structures during treatment by ultrasound releases more starch granules in the aqueous phase, resulting in exposing a much larger surface area to enzymes. Therefore, it is expected that samples subjected to ultrasound irradiation show higher reducing sugar release than the untreated samples [10a,62,77–79,85a,86,87]. In general, reduction in particle size, better mixing and the release of starch bonded to lipids that do not have access to the hydrolyzing enzyme are found as basic reasons for the success of ultrasonic pretreatment in enhancing glucose release during enzymatic hydrolysis of starch. Cavitation collapse introduced by ultrasound irradiation into the enzyme processing solution significantly enhances the transport of enzyme macromolecules toward the substrate surface of starch, leading to increase of enzymatic hydrolysis yields [62]. Ultrasonic treatment enhances starch-protein separation and subsequently increases α -amylase activity, resulting in improvement of sugar release [101,102,112].

According to Huang et al. [64], the degree of hydrolysis of cornstarch granules increased sharply as the ultrasonication time was increased from 3 min to 9 min, while from 9 min to 15 min, the degree of hydrolysis increased very slowly. Ultrasound irradiation might be effective in the amorphous regions of starch during the period of 3–9 min, while even with longer ultrasonic treatment compact crystalline regions of starch might not be easily degraded. Although many researchers [10a,89] reported similar results, Nikolic et al. [77] believe that this phenomenon is attributed to the fact that further sonication after the optimum time causes a decrease in the final glucose concentration due to enzyme inhibition caused by glucose accumulation. Similarly, Kolusheva and Marinova [113] concluded that elevated concentration of glucose could affect the α -amylase inhibition and as a result, significantly decrease the starch hydrolysis rate. In the work of Shewale and Pandit [89], ultrasound treatment of 1 min with a power of 750 W applied to sorghum starch resulted in an increased saccharification by about 8%. This increase in the percentage of saccharification was attributed to the availability of additional starch for hydrolysis due to disruption of the protein matrix (surrounding starch granules) and the amylose–lipid complex by ultrasound irradiation. A higher ultrasonic intensity than the optimum value does not improve the sugar release in the hydrolysis of starch samples with prior enzyme addition, because sonication power more than the optimum value likely causes enzyme denaturation during hydrolysis [62,79]. Aside from that, in glass chambers, an excessive motion/agitation of the water occurs with high ultrasound power, which may cause decoupling between the water and horn. This decoupling can reduce the transmission of energy/power from the horn to the water. Thus, it is expected that further increase of the amplitude continues to increase the power dissipation.

As is clear from ultrasound characteristics, the final temperature of the sonicated samples without temperature control will increase in direct proportion to the power setting and treatment time. Both sonication time and power level need to be optimized in order to obtain the maximum glucose release. The effect of

sonication temperature on hydrolysis of starches is significantly dependent on type, origin and chemical composition, as well as other process parameters employed [87]. For instance, the optimum sonication temperature was found to be in the range of 30–40 °C for hydrolysis of corn meal [62], while a temperature of 60 °C was considered as the optimum sonication temperature in pretreatment of Triticale [10a,87]. However, it is believed that an increase in reducing sugar release for all sonicated starches is not due to the thermal effect but is attributed to particle size reduction and release of starch granules from fibrous structures [78,79]. Heat generated during sonication may have no effect on reducing sugar yield unless the temperature reaches the starch gelatinization point.

Saccharification of sugary corn subjected to ultrasound waves was obtained faster than treated commodity corn under the same conditions. Thus, sugary corn can be more easily disrupted in comparison with commodity corn [79]. Enzyme added to cassava slurry prior to sonication has shown a higher sugar release compared to that after sonication, which is attributed to stimulation of enzyme activity due to the acoustic streaming effect [62,78]. However, at high power and long sonication times a decrease will occur due to denaturation/degradation of enzyme due to excessive ultrasound pretreatment and the heat generated during sonication. Ultrasonic treatment could obtain a theoretical starch conversion after 3 h of saccharification as much as a jet cooking treatment at pressure of 80 psi and temperature of 150 °C, thereby it might be evident that ultrasonication could be considered a potential alternative to jet cooking [79]. The reaction rate for the control sample (nonsonicated), based on Arrhenius kinetics presented by Khanal et al. [62], was threefold higher than that of the ultrasonic treated sample. This difference in the reaction rate is due to the fact that the final glucose concentration for the ultrasonic treated sample was much higher than that of the control sample. Thus, the ultrasonic treated sample might require more time to reach the final reaction time. Khanal et al. believe that this significant enhancement of glucose release might be attributed to the fact that no enzymes were introduced into the experiment until the final saccharification step. A comparison among total solid contents of 5%, 15%, and 25% revealed that the highest reducing sugar yield was obtained at 25% total solid level, which resulted the highest energy efficiency. At higher total solid contents, more particles might be subjected to sonication energy, which opens up cell structures and releases more starch granules for subsequent enzymatic hydrolysis [78]. It can be found from the study performed by Montalbo-Lomboy et al. [79] that the continuous flow sonication of starch is more energy efficient in sugar release compared to the batch systems. The continuous system could release approximately 24 J-equivalent sugars relative to that of the control for each 1 J of dissipated ultrasonic energy, while the batch system released only 2 J of energy [86].

From the economic viewpoint it is also recommended to keep the pretreatment time low because ultrasonic systems consume a significant amount of energy. Interestingly, this is consistent with multiple findings that short duration of pretreatment is appropriate for destroying the starch crystalline arrangement of various substrates and enhancing the glucose yield [64,78,85a,89]. Based on energy balance calculations, an increase of 2 J of sugar equivalent energy for every joule of sonication energy input was generally found. Therefore, applying ultrasound technology in starch pretreatment has the potential to significantly improve the ethanol yield and improve profitability. It seems that more investigation is needed to scale up system designs to large batch or continuous processes in order to fully realize the potential benefits of ultrasound pretreatment [87]. For instance, the power dissipation in the glass sonication chamber was 50–75% higher than that of the plastic centrifuge tube for starch treatment. The chamber geometry and the mechanical impedance of the base material

may have contributed to various levels of attenuation. However, a critical assessment of the costs and benefits may be needed because of the capital and operating costs of ultrasound treatment.

4.4. Effect of ultrasound on fermentation

By integrating an ultrasonic pretreatment even with a short processing time in ethanol production from starch, the overall ethanol yield can be significantly increased. During the fermentation of starches dextrins may be hydrolyzed through the action of glucoamylase to low molecular weight sugars, which are subsequently consumed by the yeast to produce yeast cells and ethanol [10a]. Ultrasonic pretreatment can increase the dextrose depending on sonication time and ultrasound intensity and type of starch [89]. This is attributed to the availability of additional starch for hydrolysis, which is provided by ultrasound-assisted disruption of the protein matrix (surrounding starch granules) and the amylase–lipid complex. The glucose consumption is also proportional to the results of ethanol concentration because glucose is consumed as a carbon source by the yeast and fermented to ethanol [77]. Starch-to-ethanol conversion rates from sonicated starches might also be significantly higher and the fermentation time can be reduced. Thus, ultrasonic pretreatment of starch might enhance both the overall ethanol yield and fermentation rate [85a]. Montalbo-Lomboy et al. [114], compared ultrasound pretreatment with large-scale jet cooking systems, which are currently used in commercial ethanol plants. Starch-to-ethanol conversions related to jet-cooked and sonicated corn slurry samples were obtained with yields of 74% and 71.2% of theoretical yield, respectively. Although statistical analysis showed no significant difference in starch-to-ethanol conversion between the jet cooking and ultrasonication pretreatment, energy efficiency of sonication is significantly higher than that of the jet-cooking.

The free amino nitrogen (FAN) content in triticale starch increased with ultrasound treatment in comparison to the untreated control sample. Increase in sonication temperature from 40 °C to 60 °C also caused FAN content to increase. The highest ethanol contents during the SSF process will occur when the most FAN occurs in the starch solution. FAN uptake promotes yeast growth and fermentation rate. Near the end of fermentation in the study of Pejin et al. [10a], there was a slight increase in FAN content among some of the ultrasound-pretreated samples. This can be attributed to autolysis of yeast cells, the release of peptidase enzyme from stressed yeast, or excretion of FAN from yeast cells due to degradation of protein inside the yeast cells [115]. In another study, a decrease in the number of viable yeast cells after 32 h of the SSF process caused a decrease in ethanol concentration after 48 h fermentation [87]. According to Thatipamala et al. [116], the product inhibition significantly affects the ethanol yield during the ethanol batch fermentation. In their study, *S. cerevisiae* biomass yield decreased from 0.16 g/mol to 0.03 g/mol when ethanol concentration increased from zero to 107 g/L. However, the maximum number of viable cells in ultrasound pretreated samples might be much higher compared to the untreated sample [87]. Comparing between ultrasound and microwave pretreatment, in the ultrasound pretreated sample product inhibition occurred much earlier because of a decrease in the number of viable yeast cells [77].

Sonicated samples in comparison to heat treated samples of cassava chips produced nearly 29% more ethanol yield, while combined heat and ultrasound treatment had no significant effect overall. Based on energy balances, ultrasound irradiation is also preferable to heat pretreatment because of lower energy requirements [85a]. Statistical analysis also showed no significant difference in starch-to-ethanol conversion between the jet cooking and sonication in another study by Montalbo-Lomboy et al. [114]. According to a prior assessment, the capital cost for the ultrasonic

system was 10 times higher compared to the capital cost of a hydro-cooker. However, due to the large energy requirements of hydro-cookers, a lower total overall cost was estimated for continuous ultrasonication compared to jet cooking treatment, so that ultrasonication potentially is a more economical option than jet cooking [114,117].

4.5. Effect of ultrasound on immobilized enzymes

Karimi et al. [118] optimized ultrasound-assisted transesterification of waste oil catalyzed by immobilized lipase on mesoporous silica/iron oxide magnetic core-shell nanoparticles. The immobilized lipase showed a high operational stability for conversion with only a slight loss in lipase activity after four cycles.

5. Conclusions

Global desire to reduce GHG emissions has led to a search for more sustainable and environmentally friendly energy substitutes. Lignocellulosic biomass and starch-based feedstock could prove to be renewable and abundantly available sources of sugar for fermentation and conversion into transportation fuels. High cost and low efficiency of enzymatic hydrolysis of lignocellulosic feedstocks are considered to be major impediments to ethanol production. Conversion of plant cellulose into sugars still remains an expensive and slow step, and is a substantial component of the cost of ethanol from starch. The use of ultrasound energy in enzymatic processing has beneficial effects for improving sugar and ethanol yields as well as increasing the reaction rate and reducing cost.

For cellulosic feedstocks, ultrasound irradiation is able to disrupt the cell wall leading to an increase in the accessibility of cellulase enzymes to cellulose fibers. High-power ultrasound has the potential to increase the pore volume and to reduce the crystallinity of cellulosic biomass. Ultrasound pretreatment can change the surface morphology of lignocellulose materials and enhance sugar yield in the hydrolysis process. Ultrasonic-assisted alkali pretreatment effectively destroys the intermolecular hydrogen bonding of lignocellulose, resulting in a decrease in crystallinity of cellulose. The combined use of caustic (NaOH) and ultrasound is one of the more effective pretreatments for enzymatic hydrolysis of bagasse, where more than 90% of the theoretical glucose yield is obtained within 70 h. Combining ultrasonic irradiation and ionic liquids also is an effective pretreatment of lignocellulosic materials, although the effect of this kind of treatment on the physical and chemical properties of lignin still needs further investigation.

Mechanical impacts generated by the collapse of cavitation bubbles, such as micro-jet and shock waves, provide an important benefit of exposing the surface of solid substrates to the action of enzymes. For starches, the hydrodynamic shear force introduced by ultrasound irradiation has a potential to disintegrate granules, reducing particle size by up to 20-fold and reducing suspension viscosity and molecular sizes of polymers, thereby significantly enhancing the surface area for enzyme activity. Both sonication time and delivered power need to be optimized to obtain the maximum glucose release. However even a relatively short duration of sonication has been recognized as an appropriate pretreatment for destroying the starch crystalline arrangement for various substrates and significantly enhancing the glucose yield. From an economic viewpoint, the current high initial capital and operating costs suggest more investigation into cost reduction measures and economies of scale for scale up to commercial scale batch or continuous processes in order to fully realize the potential benefits of ultrasound pretreatment.

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